ESTIMATION OF CERTAIN MUSCLE AND MUSCLE FIBER PARAMETERS IN CALVES BY COULTER COUNTER AND PHOTOMICROGRAPHIC TECHNIQUES

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CHAPTER I

THE INFLUENCE OF FREEZING MUSCLE PRE AND POST RIGOR ON SUBSEQUENT MUSCLE FIBER ENUMERATION

Introduction

The immense number of fibers in a muscle makes estimation rather than absolute enumeration the only practical way to assess total muscle fiber number. Attempting to determine total muscle fiber number by teasing out and counting the microscopic-sized threads in a bovine would probably never be accomplished in a person's life time. Even the determination of the total number of fibers in a small muscle from a rat would be impractical by the technology available today.

Techniques of estimating muscle fiber number are limited. An indirect prediction of muscle fiber number was reported for different size swine by Heagarty et al. (1973). These authors found the average width of muscle fibers were approximately the same in two lines of pigs and concluded difference in muscle size in the two lines of pigs was due to increased numbers of muscle fibers in the larger line. Maxwell et al. (1974) reported a technique in which he used the average muscle fiber volume to divide into the total muscle volume to obtain an estimate of total muscle fiber number in the Guinea pig.

Stickland and Goldspink (1973) used an "indicator" muscle in which the total muscle fiber count per cross-sectional area at a specific location was used to make comparisons between animals. Muscle fiber count per cross-sectional area at specific location(s) appears to be a reasonable method to compare muscle fiber differences among the same muscle from different animals.

The purpose of the study was examining the influence of freezing muscle pre- and post-rigor on subsequent muscle fiber enumeration. The reason for evaluating these treatments was prompted by the fact that certain chemical analyses possible in pre-rigor muscle were not possible in post-rigor muscle.

Materials and Methods

Experimental material was obtained from a Holstein calf (live weight = 50 kilograms) and a Jersey calf (live weight = 19 kilograms). Both calves were 15 days old when sacrificed.

The right and left Semitendinosus, Sartorius, lateral head of the Triceps brachii, and Longissimus dorsi (fourth rib to the third lumbar vertebra section) were removed from each animal. The weight, length and specific gravity of each muscle were measured immediately after removal from the carcass.

The right Semitendinosus, Sartorius, and lateral head of the Triceps brachii muscles were allowed to enter rigor, unrestrained, at $3^{o}C$. After the muscles appeared to be in rigor (8 hours), they were wrapped in one thickness of heavy duty aluminum foil and frozen in liquid nitrogen.

The left Semitendinosus, Sartorius and lateral head of the Triceps brachii muscles were wrapped in one thickness of heavy duty aluminum foil and frozen in liquid nitrogen. The freezing procedure was completed within one hour post-mortem. Both right and left Longissimus dorsi muscle sections were treated like the left side muscles. After initial freezing all muscles were maintained at $-20^{o}C$ until histological examination was completed.

Muscles were sectioned at three locations (25, 50 and 75% of the proximal-distal length) perpendicular to the longitudinal axis of each muscle. The periphery of each face or section end at each of the three locations was traced three times with a pencil whose lead thickness was 0.5 millimeter. Each traced area was measured with a compensating polar planimeter to the nearest one-hundredth of an inch squared and the average area was converted to millimeters squared.

A core, one-quarter inch in diameter, was taken from each end of the above sections. Distortion of the tissue structure by ice crystals had occurred during the several weeks of storage. The cores taken from muscle frozen in rigor were allowed to thaw at room temperature. Muscle tissue is restored almost completely to its pre-frozen appearance on slow thawing (Love, 1966). Cores taken from muscle frozen pre-rigor were thawed at room temperature in physiological saline. After thawing, each core was positioned on a chilled microtome chuck in O.C.T. compound and refrozen with Cryokwik.

The refrozen cores were then sectioned into 10 microns thick slices by a Slee cryostat. Tissue slices were adhered to a glass microscope slide at room temperature, stained with an aqueous solution of 1% thionin by an inclusion technique for twenty seconds then covered

with immersion oil and a glass coverslip to prevent immediate dessication.

The number of fibers counted within a 10 x 10 square ocular grid field (area = 1.08 mm^2) was determined for each slice. Muscle fibers were counted if they touched either the top or right edges of the grid. If muscle fibers touched either the bottom or left edges of the grid, they were not included. Two ocular grid fields were counted for one slice taken from each core.

Cores from the muscle frozen pre-rigor and allowed to thaw underwent a contraction process known as thaw rigor (Perry, 1950; Love, 1966; Menz, 1971). The increase in core area resulting from thaw rigor for each core was calculated and used to adjust the count per area of the thaw rigor core to that of the original pre-rigor core. The area of the tissue slice was traced three times on thick paper using illumination from an X-ray viewer. The area of the trace was cut out with a scapel and weighed to the nearest tenth of a milligram. In addition, the coring device was used to cut several "sections" of the thick paper which were measured in the same manner. The area increase was calculated by:

$$\text{Area Increase} = \frac{\text{Average thick paper weight of tissue slice}}{\text{Average thick paper weight of coring device}}$$

The count per area obtained from the thaw rigor slice multiplied by the area increase represented the count per area that should have occurred in a slice from the original core, unaffected by thaw rigor.

The data were analyzed by the Statistical Analysis System (Services, 1972). The count per area was multiplied by the cross-sectional area, at the appropriate location and end attempting to

estimate the total fiber count per cross-sectional area. The data were analyzed on two bases, (1) the adjusted count per area from a particular microscopic field and (2) the estimated total muscle fiber count per cross-sectional area. The basic analysis of variance was a split-split-plot design with all factors except animals and readings fixed.

Results and Discussion

A plot of the average muscle fiber count per field versus the variance of the muscle fiber count per field was made to determine increasing variability with increasing count. The plot was made for each muscle and treatment. No increase in variability of the count was observed as the average fiber count per microscopic field increased in any of the four muscle or treatments.

In Table I is presented the analysis of variance for muscle fiber count per field in the Semitendinosus by the microscopic technique. The treatments produced a statistically significant (OSL=.0922) difference in muscle fiber count per field in the Semitendinosus. The average for muscles frozen pre-rigor was 1393 muscle fibers per 1 mm^2 and for the muscle frozen post-rigor, 914 muscle fibers per 1 mm^2. Since the cross-sectional area at the locations selected for this experiment were larger in the post-rigor muscle than the pre-rigor muscle, the estimated total fiber count per cross-sectional area was used to analyze differences between the two treatments (Table II). The treatments were not different in estimates of total muscle fiber number (OSL=.9373). The average for muscles frozen pre-rigor was 1.055 million fibers and for muscles frozen

post-rigor, 1.049 million fibers.

Location differences in muscle fiber count per field were not statistically significant (OSL=.7434, Table I). Averages at the 25, 50 and 75% locations were 1170, 1122 and 1168 muscle fibers per 1 mm^2, respectively. The side by location interaction effect for muscle fiber count per field was not statistically significant (OSL=.5100). Location and side by location interaction results for the estimated total muscle fiber count per cross-sectional area were similar to the results obtained for the muscle fiber count per field (Table II). The location differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.3723) and the side by location interaction was not statistically significant (OSL=.5289). Averages at the 25, 50 and 75% locations were 1.102, 1.024 and 1.030 million fibers per cross-sectional area, respectively.

End differences in muscle fiber count per field were statistically significant (OSL=.0707, Table I). The average for the proximal end was 1106 muscle fibers per field compared to 1202 muscle fibers per field for the distal end. A similar result was obtained for end effects from an estimated total muscle fiber number per cross-sectional area (Table II). End differences were statistically significant (OSL=.0471). The average for the proximal end was 0.996 million fibers and 1.108 million fibers for the distal end. This difference was unexpected, since muscle fibers are long thread like structures, which in the Semitendinosus, are parallel to the longitudinal axis of the muscle and if the Semitendinosus is sectioned perpendicular to its longitudinal axis, one end of the section should contain the same number of muscle fibers as the other end. The same result would be

expected for an average muscle fiber count per field taken from either end. The significant end difference may be attributed to the low number of observations (two) for each end.

The interactions of the split-split-plot (side x end, location x end and side x location x end), were not statistically significant (OSL=.8076, OSL=.2733 and OSL=.3207, respectively, Table I). Similar results are obtained for interaction effects for estimated total muscle fiber count per cross-sectional area (Table II).

The analysis of variance for muscle fiber count per field in the Sartorius is presented in Table III. For this muscle, no statistical difference was detected between the treatments for muscle fiber count per field (OSL=.3672), albeit the averages for the treatments were 1220 and 867 muscle fibers per 1 mm^2, respectively, for the pre- and post-rigor frozen muscles. Muscles frozen post-rigor were unrestrained and consequently had a larger cross-sectional area at the "section" locations selected for this experiment compared to muscle frozen pre-rigor. Therefore, the estimated total muscle fiber count per cross-sectional area was used to assess differences between the two treatments in estimating total muscle fiber number. In Table IV is presented the analysis of variance for estimated total muscle fiber number per cross-sectional area in the Sartorius. Treatments were not statistically different for total muscle fiber number per cross-sectional area (OSL=.4483). The average number of muscle fibers in the muscles frozen per-rigor was 352 thousand and 409 thousand for the muscles frozen post-rigor.

Location differences in muscle fiber count per field were not statistically significant (OSL=.4386, Table III). The average at the

25, 50 and 75% locations were 1028, 1101 and 1001 muscle fibers per 1 mm^2. The side by location interaction was not statistically significant (OSL=.2225). The location and side by location interaction results for the estimated total muscle fiber count per cross-sectional area were similar to the results obtained for the muscle fiber count per field (Table IV). Location differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.1777) and the side by location interaction was not statistically significant (OSL=.3655). The averages at the 25, 50 and 75% locations for estimated total muscle fiber count per cross-sectional area were 424, 422 and 294 thousand muscle fibers per cross-sectional area, respectively.

End differences in muscle fiber count per field were statistically significant (OSL=.0933, Table III). The average for the proximal end was 1099 compared to 988 muscle fibers per field for the distal end. A similar result was obtained for end effects in the estimated total muscle fiber number per cross-sectional area analysis (Table IV). End differences were significant (OSL=.0688). The average for the proximal end was 399 thousand and 362 thousand for the distal end. The Sartorius was similar to the Senitendinosus in this result.

In Table III, interactions of the split-split-plot (side x end, location x end and side x location x end) were all statistically non-significant (OSL=.1623, OSL=.3910 and OSL=.6578), respectively. Similar results were obtained for interaction effects for estimated total muscle fiber count per cross-sectional area (Table IV).

In Table V is presented the analysis of variance for muscle fiber count per field in the lateral head of the Triceps brachii.

Treatment differences were not statistically significant (OSL=.2775). The average for muscles frozen pre-rigor was 1464 muscle fibers per 1 mm^2 compared to 696 muscle fibers per 1 mm^2 for muscles frozen post-rigor. Unrestrained post-rigor Triceps brachii, lateral head, assumed a rectangular form after contraction. The cross-sectional area of each location was larger in muscles frozen post-rigor compared to the muscles frozen pre-rigor. Therefore, the estimated total muscle fiber count per cross-sectional area was used to assess differences between the two treatments. In Table VI is presented the analysis of variance for estimated total muscle fiber number per cross-sectional area were statistically significant (OSL=.0264).

The average of muscles frozen pre-rigor was 812 thousand muscle fibers compared to 543 thousand for muscles frozen post-rigor.

Location differences were not statistically significant for muscle fiber count per field (OSL=.7099, Table V). Averages at the 25, 50 and 75% locations were 1173, 1054 and 1011 muscle fibers per 1 mm^2. The side by location interaction was not statistically significant (OSL=.5107). Location differences for total muscle fiber number per cross-sectional area were not statistically significant (OSL=.1651, Table VI). However, the side by location interaction was statistically significant (OSL=.0759). Since the muscle frozen post-rigor was unrestrained, the locations selected were not comparable to the locations in the muscles frozen pre-rigor on a total muscle fiber count per cross-sectional area basis. For example, the average at the 50% location in muscles frozen pre-rigor was 1.048 million muscle fibers compared to 0.057 million muscle fibers at the 50% location in muscles frozen post-rigor.

End differences were not statistically significant (OSL=.6659, Table V). The average for the proximal end was 1105 muscle fibers per 1 mm^2 and 1055 muscle fibers per 1 mm^2 for the distal end. A similar result was obtained for end effects in the estimated total muscle fiber number per cross-sectional area analysis (Table VI). End differences were not statistically significant (OSL=.7946). The average for the proximal end was 686 thousand muscle fibers and 669 thousand muscle fibers for the distal end.

Interactions of the split-split-plot were not statistically significant (OSL=.6782, OSL=.6195 and OSL=.6951, respectively, Table V). Similar results are obtained for interaction effects for estimated total muscle fiber count per cross-sectional area (Table VI).

In Table VII is the analysis of variance for muscle fiber count per field in the Longissimus dorsi. Side differences of the muscles frozen pre-rigor were not statistically significant for muscle fiber count per field (OSL=.5033). The average muscle fiber count per field for the left and right side were 2266 and 2513 muscle fibers per 1 mm^2, respectively. Estimated total muscle fibers per cross-sectional area were used to assess the difference in total muscle fiber count per cross-sectional area (Table VIII). The difference in sides for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.8592). The average for the left and right side was 1.892 million and 1.960 million estimated total muscle fibers per cross-sectional area.

Location differences in muscle fiber count per field were not statistically significant (OSL=.5150, Table VII). Averages were 2378, 2561 and 2228 muscle fibers per 1 mm^2 at the 25, 50 and 75%

locations, respectively. No statistically significant side by location interaction was present (OSL=.5366). Location differences were statistically significant for estimated total muscle fibers per cross-sectional area (OSL=.0432, Table VIII). Averages were 1.507, 2.114 and 2.157 million fibers per cross-sectional area at the 25, 50 and 75% locations. The side by location interaction was not statistically significant for estimated total muscle fiber count per cross-sectional area (OSL=.5526).

End differences in muscle fiber count per field were not statistically significant (OSL=.7614, Table VII). The average for the proximal end was 2363 muscle fibers per 1 mm^2 and 2416 muscle fibers per 1 mm^2 for the distal end. A similar result was obtained with the estimated total muscle fiber count per cross-sectional area (Table VIII). End differences were not statistically significant (OSL=.6950). The average for the proximal end was 1.902 million fibers per cross-sectional area compared to 1.950 million fibers per cross-sectional area for distal end.

The interactions of the split-split-plot (side x end, location x end and side x location x end) were not statistically significant (OSL=.5590, OSL=.2799 and OSL=.2726, respectively Table VII). Similar results were obtained for interaction effects for estimated total muscle fiber count per cross-sectional area (Table VIII).

In general, the probability of making a type II error decreases with increased sample size. Each analysis of variance has expected mean square formulas which can be used to estimate variance components. An expected mean square is the expectation of a mean square, that is, the average value over an infinity of replications. Expected mean

square formulas are important in an analysis of variance because they tell which mean squares form the F-ratio for significance testing. Moreover, the expected mean squares can be used to estimate the variance components. Each mean square is an unbiased estimate of its corresponding expected mean square. Therefore, the mean squares may be substituted for the expected mean squares to calculate an estimate of each variance component in an analysis of variance. Table IX contains the expected mean squares for the experiments.

For each muscle the mean squares were used to estimate variance components. Side, location and end components of the analysis of variance were fixed; therefore, only the error terms could be successfully minimized by increasing the sample size with animals or by taking more readings. With the rapid speed that computers are capable of performing calculations, the reduction in each error term in the model was determined. Each expected mean square of the error term can be estimated by using the variance components and dividing by the appropriate degrees of freedom. First the number of readings was varied with other factors kept constant to observe the reduction in the three error terms of the model. The error term of the split-split-plot was the only error term which was reduced.

Increasing the number of animals used, keeping other factors constant, resulted in a large decrease in all three error terms of the split-split-plot. Animals and readings were varied together and the effect on the error terms were observed. The third error term of the split-split-plot all decreased more than when animals or readings were increased alone.

Summary

Post-rigor freezing of muscle decreased the number of fibers contained within a 10 x 10 square ocular grid field when compared to muscle frozen pre-rigor. Estimated total muscle fiber count per cross-sectional area from muscle frozen post-rigor and muscle frozen pre-rigor were not statistically different in the Semitendinosus or Sartorius. When the conformation of a muscle is drastically changed, due to post-rigor freezing, as was the case with the Triceps brachii, lateral head, statistically different effects were observed in estimates of total muscle fiber count per cross-sectional area.

Simultaneous chemical analysis and muscle fiber enumeration are possible in muscles frozen pre-rigor. Each of the four muscles studied are good prospects for future investigations. However, the Sartorius and Semitendinosus had statistically significant end effects. The test which produced this effect had low power due to limited observations. A technique for decreasing the error terms was described. The experimenter can determine from the minimized error terms the number of animals and readings necessary to exert pressure on the factors examined.

CHAPTER II

MUSCLE FIBER NUMBER ESTIMATES IN CALVES BY COULTER COUNTER AND PHOTOMICROGRAPHIC TECHNIQUES

Introduction

Establishing the number of muscle fibers which comprise a muscle has become increasingly popular since research indicates muscle fiber number is genetically determined and established near birth (Luff and Goldspink,1967).

Muscle fiber number, with a few exceptions, has been examined in the muscles of small laboratory animals. Smith (1963) reported a growth strain of broilers had a greater number and slightly smaller muscle fibers at hatching than a strain of smaller Leghorn birds. Luff and Goldspink (1970) examined the total number of muscle fibers in muscles of several strains of mice and found significant strain differences. Hanrahan et al. (1973) reported increased muscle weight in mice was due to increased fiber number and diameter of muscles examined after selection for body size. Aberle and Doolittle (1976) reported the same effect as Hanrahan et al. (1973) without the increase in fiber diameter. Byrne et al. (1973) reported increased muscle weight in mice was due to increased fiber number and diameter of muscles examined after selection for body weight. Maxwell et al.

(1974) has estimated the number of muscle fibers in two Guinea pig muscles and found no change in muscle fiber number with aging. Stickland and Goldspink (1973) suggested using a small muscle in the foreleg of pigs to estimate fiber number for an "indicator" of growth characteristics. However, only a few investigators have attempted to establish the number of fibers in the large muscles of meat animals.

The microscope has been the commonly accepted instrument for estimating muscle fiber number in small muscles. However, studies with the microscope are very tedious and hence self limiting. Preferably, a new automated method would be desirable for enumerating muscle fibers in the large muscles of meat animals. In recent years, the Coulter Corporation has developed equipment which has become very popular in enumerating and sizing particles in suspension. The enumeration and sizing of muscle fibers by any technique is often plaqued by many sources and few, if any, investigators have examined the variation in muscle fiber number in the large muscles of bovine.

This study had three main objectives: (1) to develop methodology to utilize the Coulter equipment in estimating muscle fiber number in bovine muscles; (2) to determine the magnitude of relationship of the Coulter Counter and Photomicrographic techniques used in this experiment; (3) to establish the amount of variability in muscle fiber number and sources thereof in four bovine muscles.

Materials and Methods

Five dairy calves from the Oklahoma State University Dairy Herd and one calf of unknown breeding were obtained for basic research on muscle fiber enumeration. The five dairy calves were sacrificed 15

days after birth. The calf of unknown breeding was sacrificed 21 days after birth. The Longissimus dorsi, from the fourth rib to the anterior end of the transverse process of the third lumbar vertebra, Sartorius, Semitendinosus and Triceps brachii, lateral head, were removed from the right and left side of each animal. Each excised muscle was weighed and measured for length of the longitudinal axis. Length in this study is defined to be: the distance from the proximal attachment of the excised muscle to the most extreme distal end of the muscle. The length of the Longissimus dorsi was determined anterior-posterior, but for ease of subsequent references will be termed proximal-distal, along with the other muscles. Length was measured to the nearest tenth of an inch and weight was measured to the nearest gram.

Muscle Freezing

The order of muscle removal was; Semitendinosus, Sartorius, Triceps brachii, lateral head and the Longissimus dorsi. The right muscle was removed before the left muscle in each case. After the length and weight were measured, each muscle was wrapped in one thickness of aluminum foil and immersed in liquid nitrogen until frozen. The entire freezing procedure was complete before one hour post-mortem. Until histological measurements and chemical analysis were obtained, the muscles were stored in a locker maintained at $-20^{o}C$.

Sectioning and Coring Muscles

Frozen muscles were sectioned perpendicular to the proximal-distal length axis at 25, 50 and 75% of the proximal-distal using a

bandsaw. The perifery of each face of the three locations was traced three times and measured for area with a compensating polar planimeter.

For coring, each face was visually divided into six locations and a one-quarter inch diameter core sample was taken at the random location indicated by rolling a die (Figure 1). The sample was removed from the coring device and its length "sub-sectioned" so that the sample length was less than the sample diameter. The adjusted core sample was then placed in Isoton at 24°C for ten minutes or until thaw rigor was complete. Isoton is the name of a phosphate buffered saline used as a blood diluent for counting and sizing red blood cells with the Coulter Counter. The core, after thaw rigor, was then positioned in O.C.T. compound on a chilled microtome chuck and frozen with Cryokwik.

Histological Preparation of Tissue Slices

Frozen cores attached to the microtome chuck were sectioned at 20 microns on a Slee Cryostat. Tissue slices were attached to clean glass microscope slides at room temperature. The tissue slices were immediately stained in a 1% aqueous solution of thionin by an inclusion technique. The slice was covered with immersion oil and a glass coverslip to prevent dessication.

Photomicrographs of two fields were taken for each slice. An A/O microscope equipped with a universal condenser 1.25, achromatic objective lens 10 x (0.25), eyepiece 10x, with a grid located in the focal plane of the eyepiece was used. The ocular grid in the eyepiece was calibrated with a stage micrometer. A 35mm Mamiya-Sekor camera with 55mm lens and variable close-up lens was positioned on a tripod in

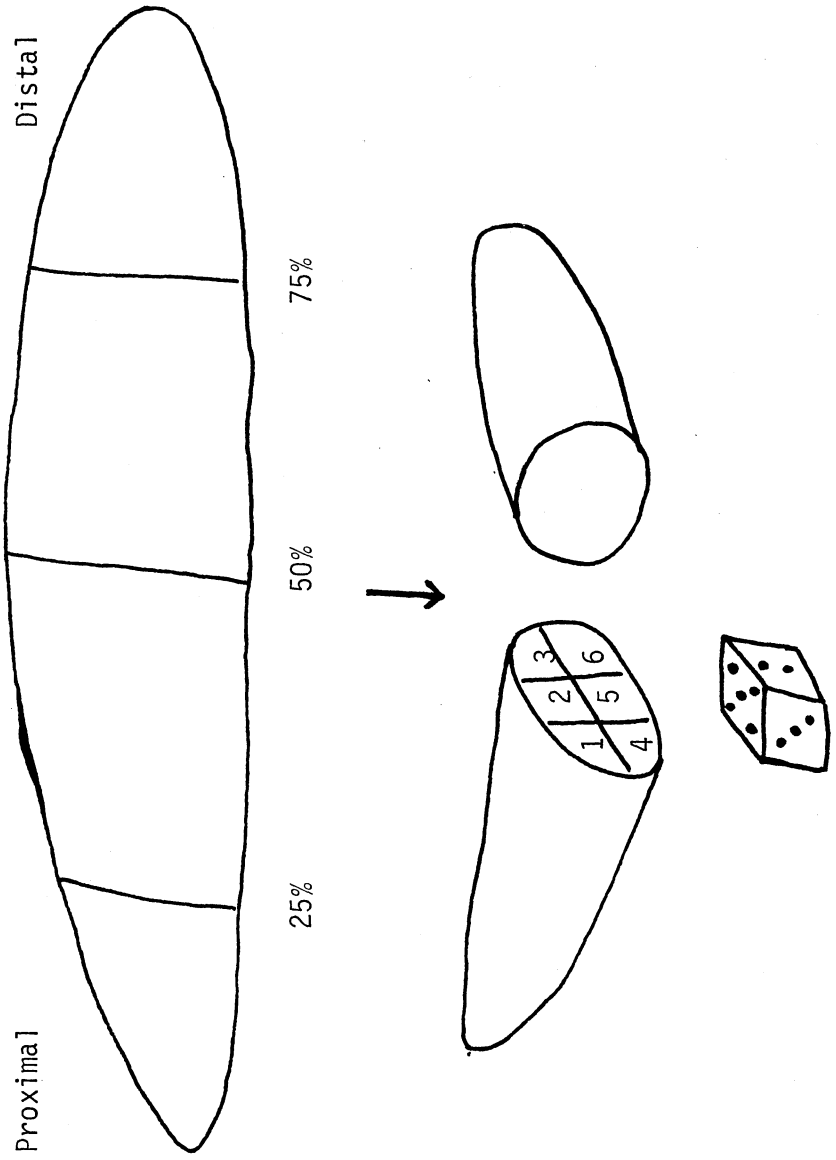


Figure 1. Sampling Procedure

front of the eyepiece with ocular grid (Figure 2). The F-stop was adjusted to the smallest value, 1.8, and both lenses of the camera were placed at infinity. The correct exposure for the shutter speed was maintained by adjusting the iris diaphragm. Only Tri-X pan film was used in this study. Film was developed and after development negatives were identified by animal, muscle, side, location, end and field (Figure 3).

Tissue Slice Preparation for the Coulter Counter

Tissue slices were sliced at 20 microns with a Slee Cryostat. Tissue slices were picked up with a loop approximately ¼ inch in diameter. Each slice was transferred with the loop to an accuvette filled with 10 ml of Isoton and allowed to stand overnight at $3^{o}C$.

Tissue slices were disrupted with a sonifier cell disruptor (Heat Systems Inc.). The sonifier horn was immersed one-half inch into the Isoton of the accuvette. The sonifier cell disruptor at an output of 70 watts for 8-10 seconds disrupted tissue slices into individual muscle fiber rods. Numerous air bubbles were present after disruption. Therefore, the accuvette vial of the small muscle fiber rods was allowed to stand 30 minutes, to dissipate air bubbles, before resuspension and counting of the muscle fibers.

Resuspention of the muscle fiber rods was accomplished by inverting the accuvette vial two or three times and sonifying with a water bath (Heat Systems Inc., 25 watt output) for 30 seconds.

Size by the Coulter Counter

A tissue slice from each muscle core was placed in an accuvette vial

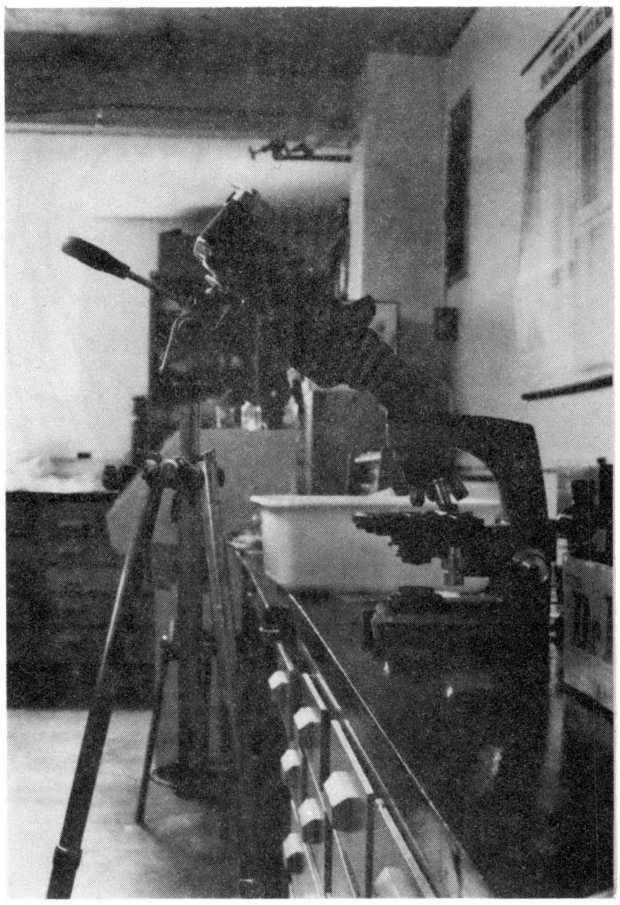


Figure 2. Microscope and Camera Outfit

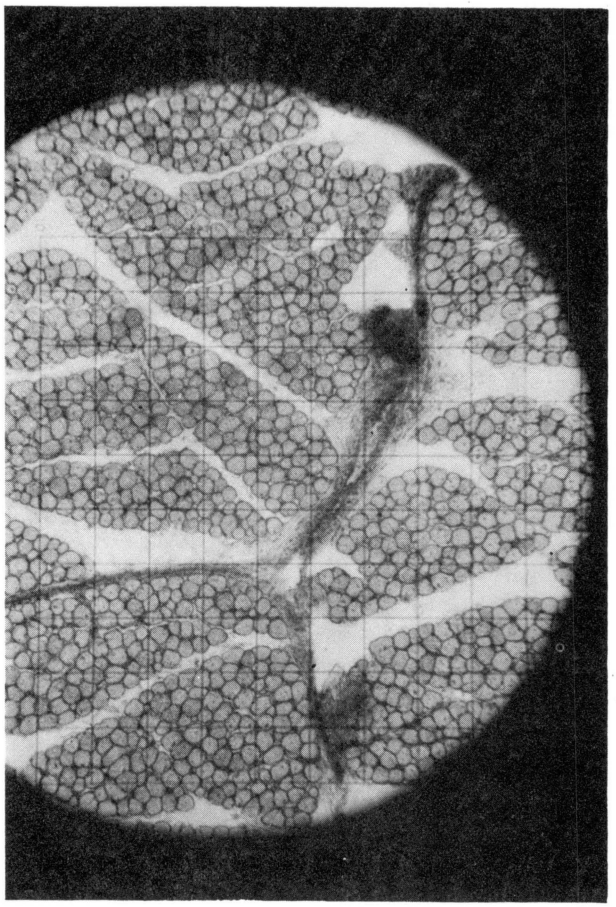


Figure 3. Identification of Film Negatives

to be disrupted and resuspended according to the procedure outlined above. The amplification and current dials of the Coulter Counter were adjusted until the mode of the fiber distribution was near channel fifty and the fiber count at the upper threshold limit was near zero on the oscilloscope screen of the Counter Channelyzer II. After determining the correct amplification and current, each vial of muscle fiber rods were sized by the Coulter Counter Channelyzer II. Particles stored in channels 0-4 were considered noise of debris and hence eliminated. Only muscle fiber rods accumulated in channels 5-99 were used in determining mean, variance and standard deviation for volume and area. Twenty to fifty thousand muscle fiber rods were sized for each distribution.

The volume of each fiber rod was obtained by multiplying the Channelyzer Channel number by the threshold factor of the Coulter Counter. The volume can be adjusted with the Coulter Channelyzer by the following equation:

$$\text{Channel No.} \times \frac{\text{W.W.}}{100} + \text{B.C.T.} \times T_f = \text{Volume in cubic microns}$$

W.W. = Window width dial setting

B.C.T. = Bace channel threshold dial setting

T_f = Amplitude x current x calibration constant of machine

Since the muscle fiber rods' volume distribution obtained with the Coulter Counter had large standard deviations, the window width dial and base channel threshold dial settings were 100 and zero, respectively.

Fiber Number by the Coulter Counter Technique

Tissue slices for count data were prepared and the correct

amplification and current dial settings for tissue slices from a muscle were established by the procedures outlined above.

Three one-half milliliter counts for each tissue slice were taken with a model ZBI Coulter Counter. To obtain the estimated number of muscle fibers for the entire tissue slice, the count was multiplied by twenty, to correct for dilution.

An average muscle fiber count per unit area was obtained from the muscle fiber count per tissue slice divided by the area of the coring device in millimeters squared. Estimated count per cross-sectional area was obtained by taking the cross-sectional area in millimeters squared and multiplying by the appropriate average muscle fiber count per millimeter squared.

Fiber Number by the Photomicrographic Technique

Photomicrographic negatives were prepared according to the procedure outlined above. Muscle fibers inside 5 x 5 squares of the ocular grid were counted for each field within a tissue slice. Muscle fibers along the top and right side of the entire square were included in the fiber count and fibers along the left and bottom side of the entire square were excluded from the muscle fiber count. The 5 x 5 squares of the ocular grid had an area of 2652.25 microns squared.

For comparison with the Coulter Counter count per tissue slice, the calculation of the tissue slice area increase was necessary to obtain a final tissue slice area for enlarging the count per ocular grid field to a count per tissue slice. Calculation of area increase is very tedious. A compensating polar planimeter will not give reliable data. Three tracings of the stained tissue slice's periphery

were made on thick paper, using a X-ray viewer for illumination. Each tracing was cut out with a scapel. The area of the coring device was cut out several times on the same thick paper. The weight of the thick paper was measured with a Mettler scale to a tenth of a milligram. Area increase was calculated by the following equation:

$$\text{Area Increase} = \frac{\text{Avg. thick paper weight tissue slice area}}{\text{Avg. thick paper weight original area}}$$

The area of the tissue slice can be calculated by the following equation:

Area of Tissue Slice = Original core area x Area Increase

A machine not available at the beginning of this research was the LiCorr area meter which will give a digital display of an area in centimeters squared. Any opaque and relatively flat surface is automatically measured by this area meter.

Count per field was normalized to represent a count per millimeter squared. The count per millimeters squared was multiplied by the area of the tissue slice, in millimeters squared, to give an estimate of the muscle fiber count per tissue slice.

Estimated count per cross-sectional area was obtained by multiplying the cross-sectional area in millimeters squared by the muscle fiber count per tissue slice divided by the area of the coring device in millimeters squared.

Statistical Analysis

The SAS computer programming system (Service, 1972) was used to analyze all data presented in this study. Each of the four muscles was considered as a seperate experiment. Therefore, no statistical comparison was made between the four muscles.

Techniques were compared on a muscle fiber count per tissue slice and estimated total muscle fiber count per cross-sectional area basis in a split-split-plot design for each muscle. Cross-product correlations for the analysis were obtained for each variable and muscle.

Total muscle fiber means for every muscle, side and location were obtained from SAS.

Results and Discussion

Selection of Sonifier Cell Disruptor Settings

Settings for the sonifier cell disruptor were determined by experimenting with different power and time settings. Selected settings and results are presented in Table X. Using a high power output for a brief period of time resulted in no visable cellular fracturing or shattering of the muscle fibers contained in a tissue slice. Low power output for a long period of time resulted in a definite cellular homogenization of the muscle fibers. Settings obtained in this experiment worked for 15 day calf muscle tissue slices 20 microns in thickness. Tissue slices from other species, mature animals, different thicknesses and chemically fixed might require different settings of the sonifier cell disruptor.

Changes Due to Thaw Rigor

Thaw rigor changed the core's original area. In Figure 4 is presented possible orientations of muscle fibers within a core. Figure 4A and 4B display the typical appearance of muscle fiber bundles in the Sartorius, Semitendinosus and Triceps brachii, lateral head

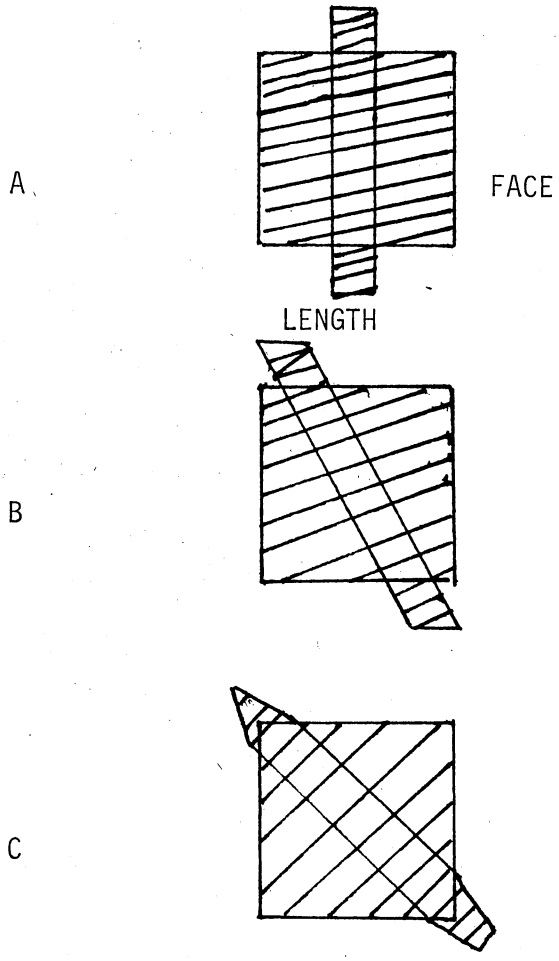


Figure 4. Possible Orientations of Fibers in Cores Before and After Thaw Rigor

before and after thaw rigor. The number of fibers in a tissue slice after thaw rigor was the same as before thaw rigor, if the length of the core was made less than the diameter of the core.

Figure 4C displays a common appearance of muscle fiber bundles in the Longissimus dorsi before and after thaw rigor. If the fibers are oriented at an angle of 45 degrees or greater to the face of the core, then more muscle fibers will appear in the face after thaw rigor than in the original core face. This geometrical argument assumes the muscle fibers lie in the lenght x face plane pictured in Figure 4. For the Sartorius, Semitendinosus and Triceps brachii, lateral head, cores were taken so that the above argument was true. However, in the Longissimus dorsi the cores were taken parallel to the length axis. Eisenhunt et al. (1965) reported a complex geometry in the Longissimus dorsi with the muscle fibers oriented at different angles from the spinous process and transverse process along the longitudinal axis of the muscle. Distortion in fiber number from reorientation due to the contraction of thaw rigor tissue was reduced by adjusting the length the length of the core to less than or equal to the diameter of the core in the Longissimus dorsi. The estimated total muscle fiber number per cross-sectional area is biased upward, since some addition of muscle fibers to the new face during thaw rigor is a definite possibility. If addition of muscle fibers was not a possibility in the Longissimus dorsi, then muscle fiber count per cross-sectional area would be based on a tissue slice parallel to the cross-sectional area of the muscle as suggested by Swatland (1975).

Estimation of Muscle Fiber Number by the Photomicrographic Technique

Longissimus dorsi. In Table XI is presented the analysis of variance for muscle fiber count per slice in the Longissimus dorsi. The muscle fiber count per slice was not statistically different in each side of the Longissimus dorsi (OSL=.2023). The average muscle fiber count per tissue slice was 95.7 thousand muscle fibers for the left side and 86.3 thousand muscle fibers for the right side. The muscle fiber count per slice was enlarged to represent the estimated total muscle fiber count per cross-sectional area (Table XII). The estimated muscle fiber count per cross-sectional area was not statistically different in each side of the Longissimus dorsi (OSL=.5739). The average estimated muscle fiber count per cross-sectional area was 2.514 million muscle fibers for the left side and 2.334 million muscle fibers for the right side.

Muscle fiber count per slice at each location was statistically significant (OSL=.0182, Table XI). The average muscle fiber count per slice at the 25, 50 and 75% locations was 1006, 848 and 875 thousand muscle fibers, respectively. Estimated muscle fiber count per cross-sectional area at each location was statistically significant (OSL=.0141, Table XII). The average estimated muscle fiber count at the 25, 50 and 75% locations was 2.092, 2.517 and 2.664 million muscle fibers per cross-sectional area. The location from which a core is removed has a significant influence on the estimate obtained for muscle fiber number per cross-sectional area.

The side x location interaction of the split-plot (Table XI) was not statistically significant for muscle fiber count per tissue slice (OSL=.0819). The side x location interaction in Table XII for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.1734).

The end from which muscle fiber count per tissue slice was taken (Table XI) was not statistically significant (OSL=.6238). The average of a tissue slice was 92.3 thousand muscle fibers for the proximal end and 89.6 thousand muscle fibers for the distal end. Estimated total muscle fibers per cross-sectional area had a similar result for end (OSL=.7918, Table XII). Average estimated muscle fiber count per cross-sectional area of the proximal end was 2.404 million muscle fibers and 2.443 million muscle fibers for the distal end of the Longissimus dorsi.

Side x end, location x end and side x location x end interactions were not statistically significant, based on a muscle fiber count per tissue slice (OSL=.9010, OSL=.8441 and OSL=.5976 respectively, Table XI). A similar result for end was observed for an estimated total muscle fiber count per cross-sectional area (Table XII).

Sartorius. Fiber counts from the left and right sides of the animal based on a count per tissue slice were not statistically significant (OSL=.3420, Table XIII). The average muscle fiber count from a tissue slice was 62.2 thousand muscle fibers for the left side and 64.4 thousand muscle fibers for the right side. Estimates of total muscle fibers per cross-sectional area were similar (Table XIV). Sides were not statistically different for estimated

total muscle fibers per cross-sectional area (OSL=.9450). The average muscle fiber count per estimated total muscle fibers per cross-sectional area was 557 thousand muscle fibers for the left side and 558 thousand muscle fibers for the right side.

Location differences for muscle fiber count per tissue slice are presented in Table XIII. Location differences were not statistically significant for muscle fiber count per tissue slice (OSL=.6786). The average muscle fiber count per slice for the 25, 50 and 75% locations were 62.5, 64.4 and 63.0 thousand muscle fibers, respectively. However, location differences existed for an estimated total muscle fiber count per cross-sectional area (OSL=.0382, Table XIV). The average estimated total muscle fibers per cross-sectional area for the 25, 50 and 75% locations were 549, 593 and 530 thousand muscle fibers.

The side x location interaction of the split-plot (Table XII) was not statistically significant for muscle fiber count per tissue slice (OSL=.6591). The side x location interaction of Table XIV for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.5167).

End differences for muscle fiber count per tissue slice are presented in Table XII. Differences in ends for muscle fiber count per tissue slice were statistically significant (OSL=.0038). The average muscle fiber count for a slice taken from the proximal end was 60.2 thousand muscle fibers and 66.4 thousand muscle fibers for the distal end. End differences were not statistically significant for estimated total muscle fibers per cross-sectional area (OSL=.0752, Table XIV). The average estimated total muscle fibers per

cross-sectional area was 542 thousand muscle fibers for the proximal end and 573 thousand muscle fibers for the distal end.

Location x end and side x location x end interactions were not statistically significant for muscle fiber count per tissue slice (OSL=.7100 and OSL=.1001, respectively, Table XIII). The side x end interaction was statistically significant for both muscle fiber count per tissue slice (OSL=.0122) and muscle fiber count per cross-sectional area (OSL=.0150), Table XIII and Table XIV, respectively. In Table XIV is presented a similar result for estimated total muscle fiber count per cross-sectional area. Location x end and side x location x end were not statistically significant (OSL=.5595 and OSL=.1663), respectively.

Semitendinosus. Muscle fiber count per tissue slice was not significantly different for each side of the Semitendinosus (OSL=.5061, Table XV). The average muscle fiber count per tissue slice was 66.9 thousand muscle fibers for the left side and 69.3 million fibers for the right side. Estimated total muscle fiber count per cross-sectional area was used to detect a difference in sides of the animal (Table XVI). Estimated total muscle fiber count per cross-sectional area for the left and right side was not statistically significant (OSL=.1087). The average estimated total muscle fiber count per cross-sectional area for the left side was 1.772 million fibers and 1.921 million fibers for the right side of the Semitendinosus.

Muscle fiber count per tissue slice at each location for the photomicrographic technique is presented in Table XV. Location

differences in muscle fiber count per tissue slice were statistically significant (OSL=.0278). Averages for muscle fiber count per tissue slice at the 25, 50 and 75% locations were 68.8, 61.9 and 73.5 thousand muscle fibers, respectively. Estimated total muscle fiber count per cross-sectional area for the locations is presented in Table XVI. Location differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.1360). Averages for estimated total muscle fiber count per cross-sectional area at the 25, 50 and 75% locations were 1.869, 1.724 and 1.945 million fibers, respectively, by the photomicrographic technique.

In Table XV, the side x location interaction for muscle fiber count per tissue slice was not statistically significant (OSL=.5928). The side x location interaction for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.2838, Table XVI).

In Table XV, end differences for muscle fiber count per tissue slice were not statistically significant (OSL=.5385). The average muscle fiber count per tissue slice from the proximal end was 69.0 thousand muscle fibers and 67.2 thousand muscle fibers for the distal end of the Semitendinosus. A similar result for end differences was obtained for the estimated total muscle fiber count per cross-sectional area (Table XVI). End differences for estimated total muscle fiber count per cross-sectional area were not significant (OSL=.5175). The average estimated muscle fiber count per cross-sectional area of the proximal end was 1.873 million muscle fibers and 1.819 million fibers for the distal end of the Semitendinosus.

In Table XV, side x end and side x location x end interactions for

muscle fiber count per tissue slice were not statistically significant (OSL=.6087 and OSL=.7020), respectively. The location x end interaction for muscle fiber count per tissue slice was statistically significant (OSL=.0201). A similar result was obtained for estimated total muscle fiber count per cross-sectional area (Table XVI). Side x end and side x location x end interactions for estimated total muscle fiber count per tissue slice were not statistically significant (OSL=.6480 and OSL=.7601), respectively. The location x end interaction for estimated total muscle fiber count per cross-sectional area was statistically significant (OSL=.0117).

Triceps brachii. Side differences for muscle fiber count per tissue slice were not statistically significant (OSL=.5247, Table XVII). The average muscle fiber count per tissue slice from the left side was 62.2 thousand muscle fibers and 65.4 thousand-muscle fibers for the right side of the Triceps brachii lateral head. Estimated total muscle fiber count per cross-sectional area was similar (Table XVIII). Side differences for estimated total muscle fibers per cross-sectional area were not statistically significant (OSL=.5798). The average estimated total muscle fiber count per cross-sectional area was 1.080 million fibers for the left side and 1.130 million fibers for the right side of the Triceps brachii, lateral head.

In Table XVII, location differences in muscle fiber count per tissue slice were not statistically significant (OSL=.9780). Averages for muscle fiber count per tissue slice at the 25, 50 and 75% locations were 63.4, 63.6 and 64.3 thousand muscle fibers, respectively.

Results were not similar for estimated total muscle fiber count per cross-sectional area, Table XVIII. Location differences for estimated total muscle fiber count per cross-sectional area were statistically significant (OSL=.0001). Averages for estimated total muscle fiber count per cross-sectional area at the 25, 50 and 75% locations were 1.161, 1.383 and 0.771 million muscle fibers, respectively.

The side x location interaction for muscle fiber count per tissue slice was not statistically significant (OSL=.5652, Table XVII). In Table XVIII, the side x location interaction for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.6613).

End differences for muscle fiber count per tissue slice are presented in Table XVII. Differences in muscle fiber count per tissue slice from the proximal and distal ends were not statistically significant (OSL=.5247). The average muscle fiber count per tissue slice from the proximal ends was 62.7 thousand muscle fibers and 64.8 thousand muscle fibers for the distal ends. A similar result for end differences was obtained for the estimated total muscle fiber count per cross-sectional area, Table XVIII. End differences in estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.9501). The average estimated total muscle fiber count per cross-sectional area for the proximal ends was 1.107 million muscle fibers and 1.103 million fibers for the distal ends.

Interactions of the split-split-plot are presented in Table XVII for muscle fiber count per tissue slice. Side x end, location x end and side x location x end interactions for muscle fiber count per tissue slice were not statistically significant (OSL=.8239, OSL=.6461

and OSL=.5398), respectively. A similar result for the interactions was observed for estimated total muscle fiber count per cross-sectional area, Table XVIII.

Estimation of Muscle Fiber Number by the Coulter Counter Technique

Longissimus dorsi. In Table XIX is presented the analysis of variance for muscle fiber count per slice in the Longissimus dorsi. Side differences in muscle fiber count per slice were not statistically significant (OSL=.2624). The average muscle fiber count per slice was 71.6 thousand muscle fibers for the left side and 67.4 thousand muscle fibers for the right side by the Coulter Counter technique. The muscle fiber count per slice was enlarged to represent the estimated total muscle fiber count per cross-sectional area in the Longissimus dorsi, Table XX. Side differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.5474). The average estimated total muslce fiber count per cross-sectional area was 1.894 million muscle fibers for the left side and 1.800 million muscle fibers for the right side of the Longissimus dorsi.

Location differences in muscle fiber count per tissue slice are presented in Table XIX. Location differences in muscle fiber count per tissue slice were not statistically significant (OSL=.1847). Averages for muscle fiber count per tissue slice at the 25, 50 and 75% locations were 73.3, 64.8 and 70.5 thousand muscle fibers, respectively. Location differences for estimated total muscle fiber

count per cross-sectional area were statistically significant (OSL= .0015), Table XX. Averages for estimated total muscle fibers per cross-sectional area at the 25, 50 and 75% locations were 1.511, 1.923 and 2.108 million muscle fibers, respectively.

In Table XIX, the side x location interaction for muscle fiber count per tissue slice was not statistically significant (OSL=.5622). A similar interaction result was obtained for the estimated total muscle fiber count per cross-sectional area, Table XX. The side x location interaction for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.3392).

End differences for muscle fiber count per tissue slice were not statistically significant (OSL=.9436, Table XIX). The average muscle fiber count per tissue slice was 69.7 thousand muscle fibers for the proximal ends and 69.4 thousand muscle fibers for the distal ends. A similar result for ends was obtained for estimated total muscle fiber count per cross-sectional area (Table XX). End differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.9126). The average estimated total muscle fiber count was 1.854 million muscle fibers for the proximal ends and 1.841 million muscle fibers for the distal ends of the Longissimus dorsi.

Interactions of the split-split-plot are presented in Table XIX for muscle fiber count per tissue slice by the Coulter Counter technique. Side x end, location x end and side x location x end interactions for muscle fiber count per tissue slice were not statistically significant (OSL=.6380, OSL=.5571 and OSL=.8219), respectively. A similar result for the interactions was observed for

the variable estimated total muscle fiber count per cross-sectional area (Table XX).

Sartorius. In Table XXI is presented the analysis of variance for muscle fiber count per tissue slice in the Sartorius by the Coulter Counter technique. Differences in sides for muscle fiber count per tissue slice were not statistically significant (OSL=.5269). The average muscle fiber count per tissue slice was 48.5 thousand muscle fibers for the left side and 46.9 thousand muscle fibers for the right side by the Coulter Counter technique. Muscle fiber count per tissue slice was enlarged to represent estimated total muscle fiber count per cross-sectional area (Table XXII). Side differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.2926). The average estimated total muscle fiber count per cross-sectional area was 445 thousand muscle fibers for the left side and 421 thousand muscle fibers for the right side of the Sartorius.

Location differences for muscle fiber count per tissue slice in the Sartorius by the Coulter Counter technique are presented in Table XXI. Location differences for muscle fiber count per tissue slice were not statistically significant (OSL=.3795). Averages for muscle fiber count per tissue slice at the 25, 50 and 75% locations were 46-6, 49.2 and 47.2 thousand muscle fibers, respectively. Location differences for estimated total muscle fiber count per cross-sectional area were statistically significant (OSL=.0513, Table XXII). Averages for estimated total muscle fibers per cross-sectional area

at the 25, 50 and 75% locations were 423, 468 and 408 thousand muscle fibers, respectively.

In Table XXI, the side x location interaction for muscle fiber count per tissue slice was not statistically significant (OSL=.3823). A similar interaction result was obtained for an estimated total muscle fiber count per cross-sectional area (Table XXII). The side x location interaction for an estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.5949).

End differences for muscle fiber count per tissue slice are presented in Table XXI for the Sartorius by the Coulter Counter technique. End differences for muscle fiber count per tissue slice were not statistically significant (OSL=.1117). The average muscle fiber count per tissue slice from the proximal ends was 46.4 thousand muscle fibers from the proximal ends and 48.9 thousand muscle fibers from the distal ends. A similar result for ends was obtained for estimated total muscle fiber count per cross-sectional area (Table XXII). End differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.5687). The average estimated total muscle fiber count per cross-sectional area was 438 thousand muscle fibers for the distal ends and 428 thousand muscle fibers for the proximal ends of the Sartorius by the Coulter Counter technique.

Interactions of the split-split-plot are presented in Table XXI. Side x end, location x end and side x location x end interactions for muscle fiber count per tissue slice were not statistically significant (OSL=.7108, OSL=.5174 and OSL=.0745), respectively. Interaction effects were similar for estimated total muscle fiber count per cross-sectional area (Table XXII).

Semitendinosus. An analysis of variance for muscle fiber count per tissue slice for the Semitendinosus by the Coulter Counter technique is presented in Table XXIII. The difference in sides for muscle fiber count per tissue slice was not statistically significant (OSL=.5348). The average muscle fiber count per tissue slice was 52.5 thousand muscle fibers for the left side and 54.2 thousand muscle fibers for the right side. The muscle fiber count per tissue slice was enlarged to represent an estimated total muscle fiber count per cross-sectional area (Table XXIV). The difference in sides for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.2012). The average estimated total muscle fiber count per cross-sectional area was 1.385 million fibers for the left side and 1.491 million fibers for the right side.

Location differences for muscle fiber count per tissue slice in the Semitendinosus by the Coulter Counter technique are presented in Table XXIII. Location differences for muscle fiber count per tissue slice were not statistically significant (OSL=.1406). Averages for muscle fiber count per tissue slice at the 25, 50 and 75% locations were 55.5, 49.5 and 55.0 thousand muscle fibers, respectively. In Table XXIV, location differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.3527). Averages for estimated total muscle fiber count per cross-sectional area at the 25, 50 and 75% locations were 1.498, 1.379 and 1.436 million muscle fibers, respectively.

In Table XXIII, the side x location interactions for muscle fiber

count per tissue slice was not statistically significant (OSL=.8531). A similar interaction result was obtained for an estimated total muscle fiber count per cross-sectional area (Table XXIV). The side x location interaction was not statistically significant (OSL=.9166).

End differences for muscle fiber count per tissue slice are presented in Table XXIII for the Semitendinosus by the Coulter Counter technique. End differences for muscle fiber count per tissue slice were not statistically significant (OSL=.6047). The average muscle fiber count per tissue slice was 53.7 thousand muscle fibers for the proximal ends and 52.9 thousand muscle fibers for the distal ends. A similar result for ends was obtained for an estimated total muscle fiber count per cross-sectional area (Table XXIV). End differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.5230). The average estimated total muscle fiber count per cross-sectional area was 1.453 million muscle fibers for the proximal end and 1.422 million muscle fibers for the distal end of the Semitendinosus.

Interactions of the split-split-plot are presented in Table XXIII. Side x end and side x location x end interactions for muscle fiber count per tissue slice were not statistically significant (OSL=.7486 and OSL=.6355), respectively. The location x end interaction for muscle fiber count per tissue slice was statistically significant (OSL=.0338). A similar result for interactions of the split-split-plot was obtained for an estimated total muscle fiber count per cross-sectional area (Table XXIV). Side x end and side x location x end interactions for an estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.6770 and

OSL=.8018), respectively. The location x end interactions for an estimated total muscle fiber count per cross-sectional area was statistically significant (OSL=.0493).

Triceps Brachii. An analysis of variance for muscle fiber count per tissue slice for the Triceps brachii, lateral head by the Coulter Counter technique is presented in Table XXV. The difference in sides for muscle fiber count per tissue slice was not statistically significant (OSL=.7535). The average muscle fiber count per tissue slice was 48.9 thousand muscle fibers for the left side and 48.0 thousand muscle fibers for the right side. Muscle fiber count per tissue slice was enlarged to represent an estimated total muscle fiber count per cross-sectional area (Table XXVI). The difference in sides for an estimated total muscle fiber number per cross-sectional area was not statistically significant (OSL=.9260). The average estimated total muscle fiber count per cross-sectional area was 834 thousand muscle fibers for the left side and 829 thousand muscle fibers for the right side.

Location differences for muscle fiber count per tissue in the Triceps brachii, lateral head by the Coulter Counter technique are presented in Table XXV. Location differences for muscle fiber count per tissue slice were not statistically significant (OSL=.8306). Averages for muscle fiber count per tissue slice at the 25, 50 and 75% locations were 49.3, 47.5 and 48.5 thousand muscle fibers, respectively. In Table XXVI, location differences for estimated total muscle fibers per cross-sectional area were statistically significant (OSL=.0001). Averages for estimated total muscle fiber count per cross-sectional

area at the 25, 50 and 75% locations were 0.907, 1.016 and 0.571 million muscle fibers, respectively.

In Table XXV, the side x location interaction for muscle fiber count per tissue slice was not statistically significant (OSL=.5681). A similar interaction result was obtained for an estimated total muscle fiber count per cross-sectional area (Table XXVI). The side x location interaction for estimated total muscle fiber count per cross-sectional area was not statistically significant (OSL=.5850).

End differences for muscle fiber count per tissue slice are presented in Table XXV for the Triceps brachii, lateral head by the Coulter Counter technique. End differences for muscle fiber count per tissue slice were not statistically significant (OSL=.8149). The average muscle fiber count per tissue slice was 48.7 thousand muscle fibers for the proximal end and 48.2 thousand muscle fibers for the distal end. A similar result for ends was obtained for an estimated total muscle fiber count per cross-sectional area (Table XXVI). End differences for estimated total muscle fiber count per cross-sectional area were not statistically significant (OSL=.6820). The average estimated total muscle fiber count per cross-sectional area was 822 thousand muscle fibers for the distal end and 840 thousand muscle fibers for the proximal end.

Interactions of the split-split-plot are presented in Table XXV. Side x end, location x end and side x location x end interactions for muscle fiber count per tissue slice were not statistically significant (OSL=.7864, OSL=.7464 and OSL=.2900), respectively. A similar result for interactions of the split-split-plot was obtained for an estimated total muscle fiber count per cross-sectional area (Table XXVII).

Different components of variance had different levels of significance by technique and muscle for muscle fiber count per tissue slice. Ends were statistically significant for muscle fiber count per tissue by the photographic technique in the Sartorius. This result suggests that tissue slices taken from one end of a core from a particular location have more fibers than tissue slices taken from the opposite end. No logical explanation can be offered for this apparent result.

Different components of variance had different levels of significance by technique and muscle for estimated total muscle fiber number per cross-sectional area. Location was statistically significant in all muscles for the Semitendinosus. The three locations of the Semitendinosus were not statistically different for estimated total muscle fiber number per cross-sectional area by either technique.

Correlation Between the Coulter Counter Technique and Photomicrographic Technique

A table of Expected Mean Squares for the split-split-plot design is presented in Table XXVII to facilitate understanding of the Cross Products analysis.

Correlations were obtained for selected variance components from the cross products analysis for Coulter and Photomicrographic muscle fiber count per tissue slice in the Longissimus dorsi, Table XXVIII. A point scatter plot of Coulter versus Photomicrographic estimates of muscle fiber count per slice is presented in Figure 5.

These data suggest a strong positive relationship between the Coulter count and Photomicrographic count in the Longissimus dorsi.

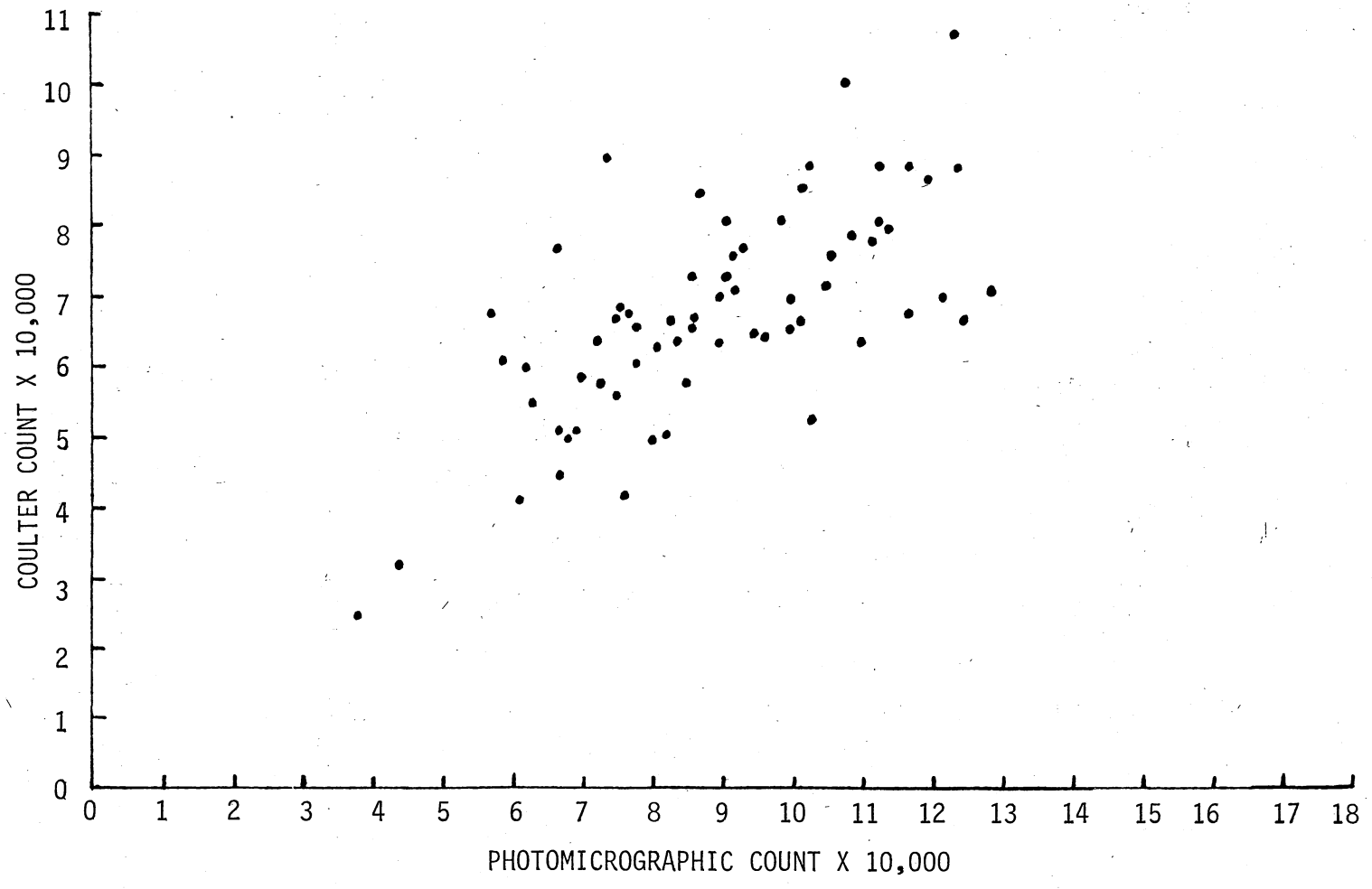


Figure 5. Point Scatter Plot of the Count per Tissue Slice by Coulter Counter versus Photomicrographic Techniques in the Longissimus dorsi

However, the photomicrographic count was usually larger than the Coulter count. The variability of animals was expected to be large; therefore, the six points for animals in this analysis were expected to be widely spread and highly correlated ($r=.834$; $P \leq .05$). Side and end had only two points and the correlation between two points is always perfect. The term which contained all the variability was the error term of the split-split-plot. The error term of the split-split-plot was used to assess the significance of the relationship between Coulter count and photomicrographic count. 'A X E + A X E X S/L' is the error term of the split-split-plot and the correlation coefficient was statistically significant ($r=.864$; $P < .01$).

Correlations for selected variance components from the cross products analysis for Coulter and photomicrographic muscle fiber count per tissue slice in the Sartorius are presented in Table XXIX. A point scatter plot of Coulter versus Photomicrographic estimates of muscle fiber count per slice is presented in Figure 6. The Counter count and photomicrographic count showed a positive relationship in the Sartorius; however, the correlation coefficient of the error term of the split-split-plot, 'A X E + A X E X S/L', was not statistically significant ($r=.267$, $P > .05$). The two counting procedures were expected to be related. However, the assumptions of the cross products analysis may not have been met, as the individual plots of the points from each animal revealed that the covariance of Coulter count and photomicrographic count were not equal between the animals in the Sartorius.

Correlations were obtained for selected variance components from the cross products analysis for Coulter and Photomicrographic muscle

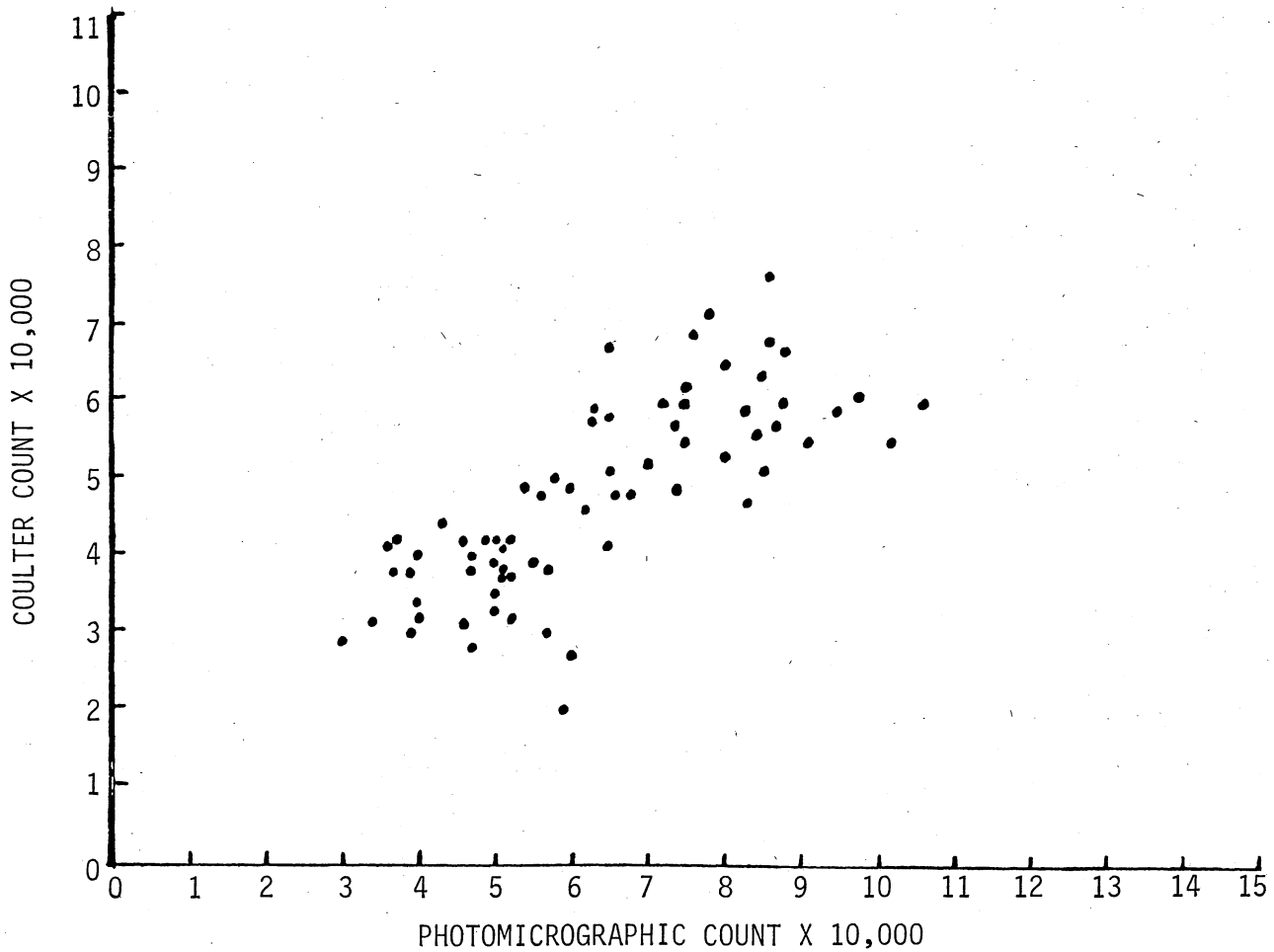


Figure 6. Point Scatter Plot of the Count per Tissue Slice by Coulter Counter versus Photomicrographic Techniques in the Sartorius

fiber count per tissue slice in the Semitendinosus, Table XXX. A point scatter plot of Coulter versus photomicrographic estimates of muscle fiber count per slice is presented in Figure 7. These data suggest a strong positive relationship between the Coulter count and photomicrographic count in the Semitendinosus. However, the photomicrographic count was usually larger than the Coulter count. The correlation coefficient of the error term of the split-split-plot, 'A X E + A X E X S/L', was statistically significant ($r=.528$; $P < .05$).

Correlations were obtained for selected variance components from the cross products analysis for Coulter and photomicrographic muscle fiber count per tissue slice in the Triceps brachii, lateral head, Table XXXI. A point scatter plot of Coulter versus photomicrographic estimates of muscle fiber count per tissue slice is shown in Figure 8. The Coulter count and photomicrographic count showed a positive relationship in the Triceps brachii, however, the correlation coefficient of the error term of the split-split-plot, 'A X E + A X E X S/L', was not statistically significant ($r=.237$; $P > .05$). The two counting procedures were expected to be related. However, the assumptions of the cross products analysis may not have been met, as the individual plots of the points from each animal revealed that the covariance of Coulter count and photomicrographic count were not equal between the animals in the Triceps brachii, lateral head.

Muscle fiber count per tissue slice was enlarged to represent an estimated total muscle fiber count per cross-sectional area for each muscle. Correlations for selected variance components from the cross products analysis for Coulter and photomicrographic estimation of total muscle fiber number per cross-sectional area are presented in

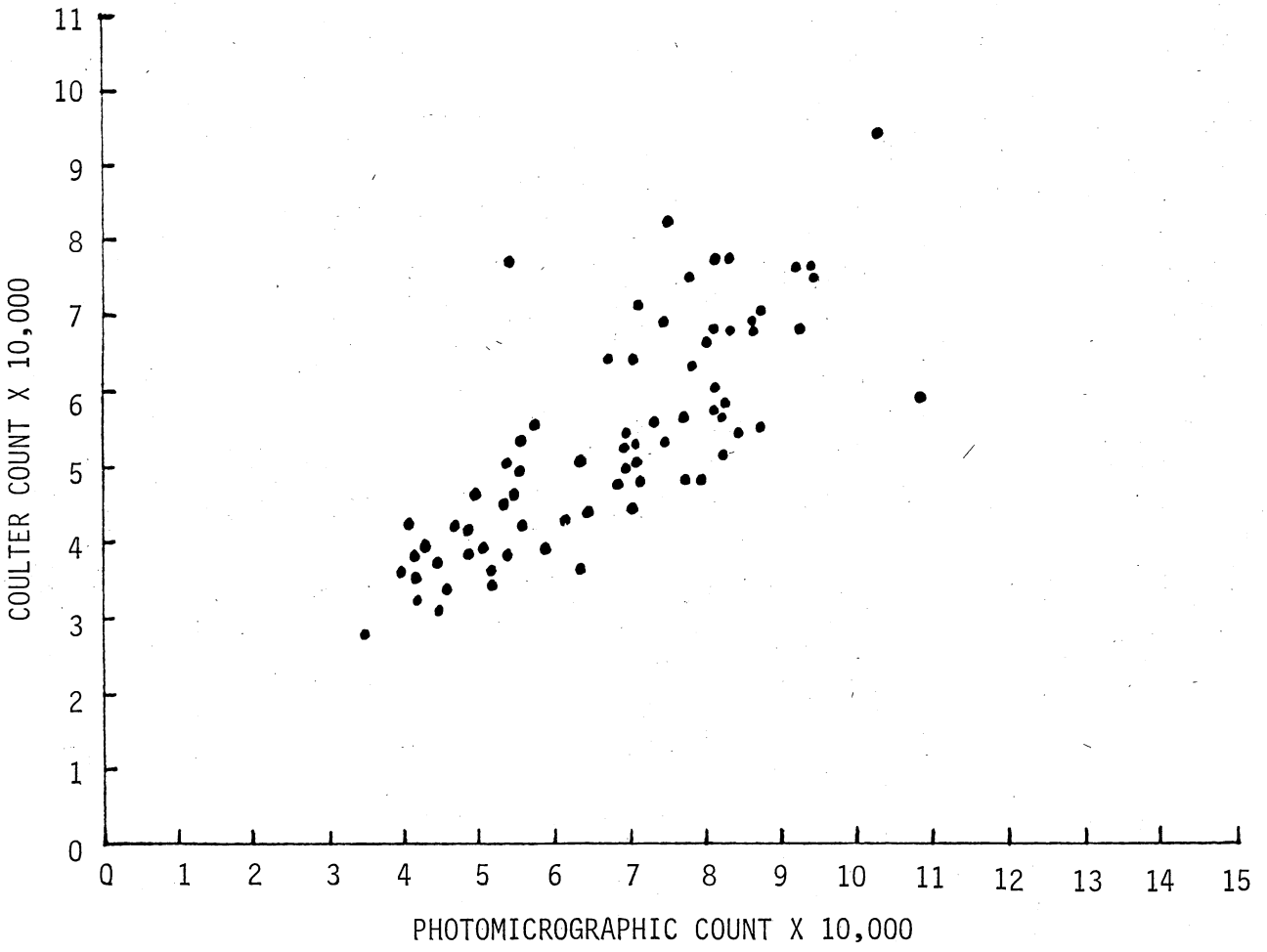


Figure 7. Point Scatter Plot of the Count per Tissue Slice by Coulter Counter versus Photomicrographic Techniques in the Semitendinosus

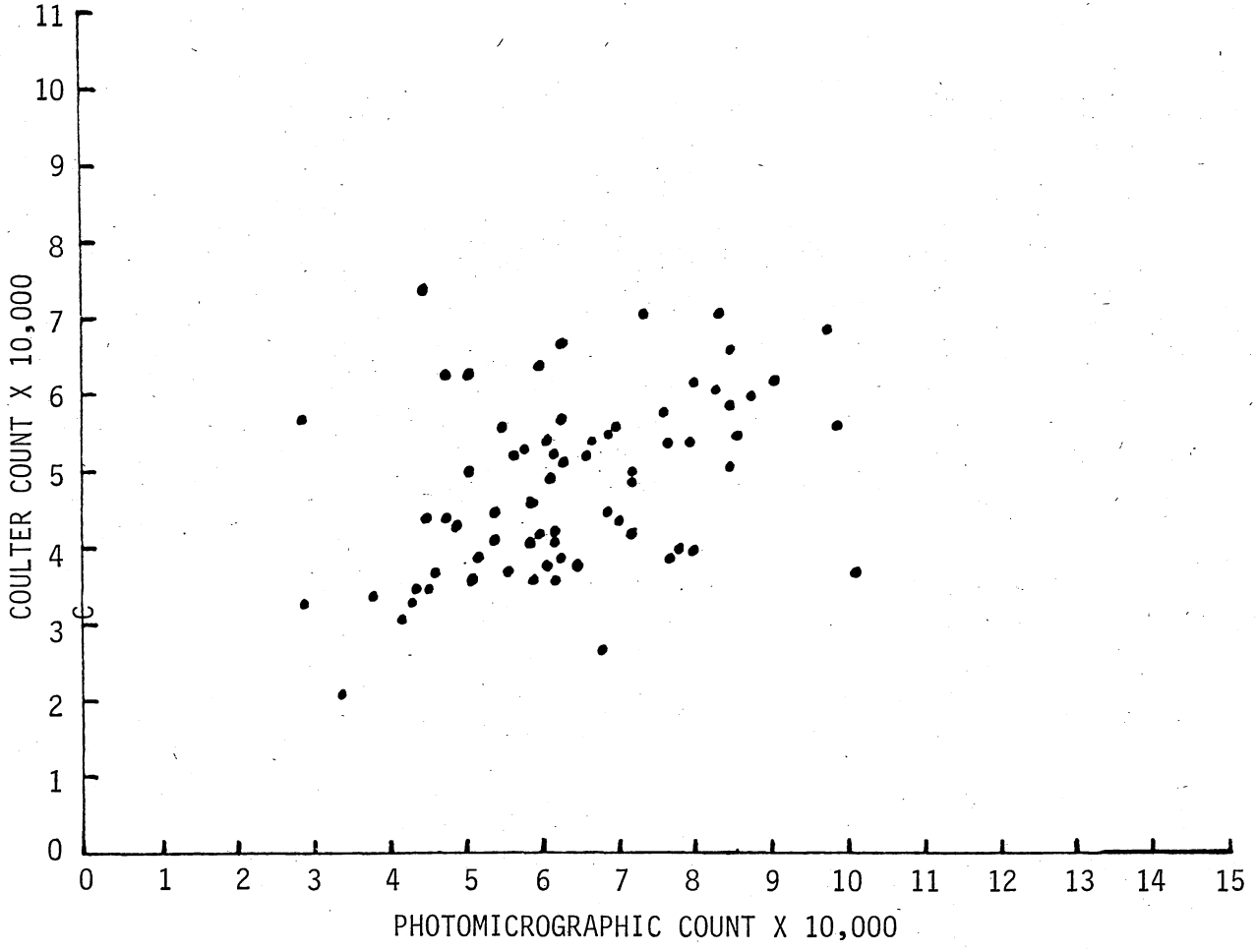


Figure 8. Point Scatter Plot of the Count per Tissue Slice by Coulter Counter versus Photomicrographic Techniques in the Triceps brachii

Tables XXXII, XXXIII, XXXIV and XXXV for the Longissimus dorsi, Sartorius, Semitendinosus, and Triceps brachii, respectively. These results suggested that the correlation coefficients between the two enumeration procedures were changed when enlarged to represent the total count per cross-sectional area.

Muscle Fiber Number per Cross-Sectional Area

In Tables XXXVI, XXXVII, XXXVIII and XXXIX are presented the mean and standard deviation of muscle fiber counts at each location and side of the animal in the Longissimus dorsi, Sartorius, Semitendinosus and Triceps brachii, respectively. These results showed that the estimated total muscle fiber number per cross-sectional area was larger for the photomicrographic technique than for the Coulter counter technique. However, as indicated by these results and the cross products analyses data both techniques yield similar results. The number of fibers at a single location is highly variable. If more cores had been taken for each location, better results may have been obtained.

In Tables XXXVI, XXXVII, XXXVIII and XXXIX the estimated mean number of fibers for a Holstein calf was larger than a Jersey calf. Charles et al. (1976) reported that muscle weight distribution does not vary greatly between breeds in the four muscles used for this experiment. However, larger breeds have more total muscle weight. A logical assumption would be the parenchyma of muscle, the muscle fiber, is increased in number in the larger breeds. The means of the above tables appear to support this assumption since the two Holsteins' average muscle fiber number at a single location was numerically larger than the average of the two Jersey calves. The number of

muscle fibers within a given muscle of different calves is seen to be highly variable. Bendall and Voyle (1967) cited by Hegarty (1971) has shown muscle fiber number variation in cattle.

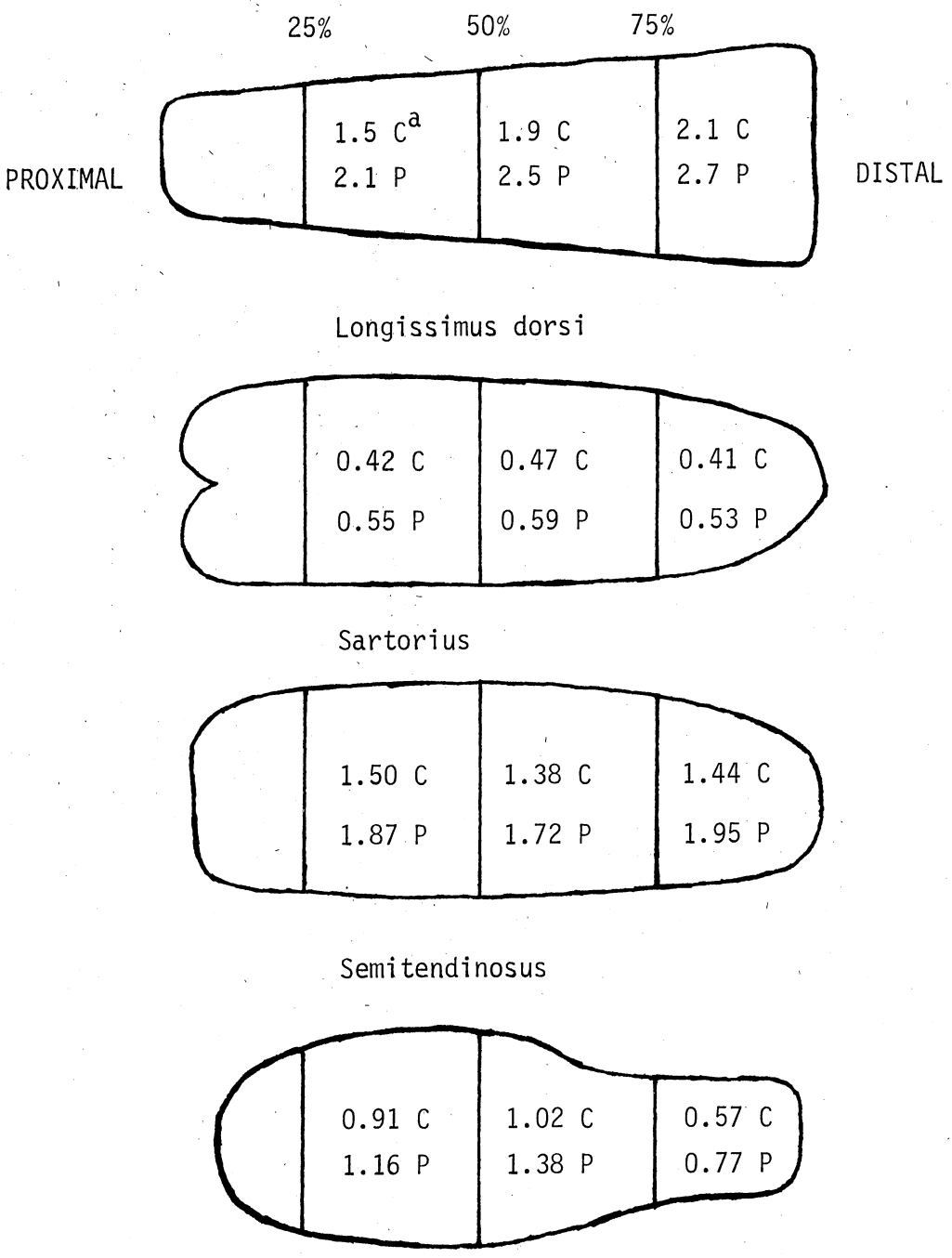
With the analysis of variance, tests can be performed to assess statistical differences between animals. However, animals were used as a blocking factor of the split-split-plot design and were assumed to be different by using the design. Therefore, no tests of differences between animals are presented. Only the statistics of the individual animals are presented which are numerically different.

In Figure 9 is presented the average number of muscle fibers expected at the three cross-sectional area locations by the Coulter Counter technique and the photomicrographic technique.

Conclusions

The components of variance in the analysis had different levels of significance by count per slice and cross-sectional area technique and muscle. The Sartorius had a significant end effect for muscle fiber count per tissue slice. Since this source of variability is present, the Sartorius is not recommended for studies involving muscle fiber number. The other muscles examined had sources of variability that should be considered before undertaking muscle fiber number estimation.

Correlations between the two methods was assessed by using the error term of the split-split-plot. A statistically significant correlation between the Coulter Counter technique and the photomicrographic technique was found for the Longissimus dorsi and Semitendinosus. However, the Sartorius and Triceps brachii, lateral



C=Coulter Counter Technique
P=Photomicrographic Technique
a=Muscle Fiber Number in Millions

Figure 9. Estimates of the Mean Number of Muscle Fibers at the three Locations of the Longissimus dorsi, Sartorius, Semitendinosus and Triceps brachii,lateral head

head were not found to show a significant relationship between the Coulter counter technique and the photomicrographic technique estimates of muscle fiber number per tissue slice.

Animals were found to exhibit a large variability in muscle fiber number at a specific location. The breed of an animal though not tested appeared to have a significant influence on the number of muscle fibers in a specific cross-sectional location. The number of fibers at a given location for the two Holstein calves was greater than the two Jersey calves. Average estimates of muscle fiber number at each location of the four muscles was larger for the photomicrographic technique than the Coulter Counter technique. However, results from the two techniques are similar when used for making comparisons.

CHAPTER III

ESTIMATION OF MUSCLE FIBER AREA IN CALVES BY COULTER COUNTER AND PHOTOMICROGRAPHIC TECHNIQUES

Introduction

Muscle fiber sizing has been the object of considerable research effort. Presently, muscle fiber diameter is the most popular method of assessing fiber size. The diameter can be measured from transverse sections in several different ways. The Zeiss particle analyzer can be used to measure a mean cross-sectional diameter (Miller, 1975). A simpler method just measures the diameter or the minimum dimension of the muscle fiber in cross-section (Livingston et al., 1966; Stickland and Goldspink, 1973). Another popular method of assessing muscle fiber diameter is through using a projection diameter of separated muscle fibers (Joubert, 1956; Tuma et al., 1962). Photomicrographic measurement of muscle fiber area from transverse sections of muscle tissue slices has been a popular technique (Montgomery, 1965 and Chrystal, 1967).

The above methods all employ the microscope for making measurements. Using the microscope is not without error. Error by the microscope depends on the operator's eyesight and the ability to focus the specimen with the microscope. Probably the greatest drawback of

the microscope is that working with the microscope is tedious and hence self-limiting.

The Coulter Counter has been used successfully to measure the particle size distribution of many different materials (Allen, 1972). Particle sizing by the Coulter Counter is automated, rapid and non-tedious. Thus the Coulter Counter could prove to be a valuable research tool if procedures could be developed to utilize the Coulter equipment in sizing muscle cells.

Therefore, a technique using the Coulter Counter was developed and the relationship of the Coulter Counter technique with a microscopic technique was examined for muscle fibers.

Materials and Methods

General procedures for obtaining experimental material and processing muscle tissue was the same as that outlined in Chapter II of this thesis.

Estimation of Muscle Fiber Area by the Coulter Counter

A tissue slice from each of the 6 cores, as previously outlined, was placed in a single accuvette vial filled with isoton. Therefore, muscle fiber area was estimated from the pooled muscle fiber tissue slices within each muscle. The amplification and current dials of the Coulter Counter for each accuvette vial were adjusted until the mode of the muscle fiber volume distribution was near channel 50 of the Coulter Channelyzer II oscilloscope screen. After determining the correct amplification and current, each vial of muscle fiber rods was

sized by the Coulter Counter Channelyzer II. Particles stored in channels 0-4 were considered noise or debris and hence eliminated. Only muscle fiber rods accumulated in channels 5-99 were used in determining mean, variance and standard deviation for volume and area. Twenty to fifty thousand muscle fiber rods were sized for each distribution.

None of the more specialized adjustments of the Coulter Counter or Coulter Channelyzer II were used in this experiment. A detailed explanation of the Coulter Principle and function of the operating components can be found in the Coulter manuals and the following articles: Allen (1972), Zalodeck (1961) and Katchel (1976).

Estimation of Muscle Fiber Area by the Photomicrographic Technique

Tissue slices for this procedure were obtained from the identical core, end and location within the muscle as for the Coulter Counter. Photomicrographic negatives of muscle fiber fields within a tissue slice were prepared by the procedures outlined in Chapter II. One square of the ocular grid and five muscle fibers, which occurred along a straight line in the photomicrographic negative, were projected and traced on paper. The traced muscle fibers and ocular grid square were measured with a compensating polar planimeter. Five muscle fibers were traced from two fields for each slice, resulting in a total of ten muscle fibers measured for each tissue slice. Six tissue slices were taken from the muscle, resulting in 60 muscle fiber areas measured per muscle.

Converting the measurements to the correct area for each muscle

fiber was accomplished by dividing the known area of the ocular grid square by the measured ocular grid square to obtain a correction factor. The correction factor was multiplied by the measured area of the muscle fiber to obtain an estimate of the actual muscle fiber area in microns squared.

Statistical Analysis

The average muscle fiber area for each animal, side and technique was analysed by the SAS computer programming system (Service, 1972).

A split-plot analysis was performed for each of the four muscles studied. The animal x side interaction was used to test side for difference in muscle fiber area. The split-plot animal x method interaction was used to test the difference in the two techniques and the animal x side x method interaction was used to test the side x methods interaction.

To assess the overall relationship between the two techniques a split-split-plot design was used, in which muscles served as the main plot treatments. The animal x muscle interaction was used to test muscle differences in the main plot. The animal x side interaction and animal x muscle x side interaction were used to test side and muscle x side. In the split-split-plot, the animal x method, animal x muscle x method, animal x side x method and animal x muscle x side x method interactions were used to test method, muscle x method, side x method and muscle x side x method, respectively.

A cross products analysis was performed to place a numerical value on the relationship existing between the techniques in each muscle.

Results and Discussion

In Tables XL, XLI, XLII and XLIII are presented the means and standard deviations for muscle fiber area in the Longissimus dorsi, Sartorius, Semitendinosus and Triceps brachii, respectively. A comparison of the muscle fiber area means, in the four muscles, between the two techniques revealed that the muscle fiber area obtained with the Photomicrographic technique was consistently larger than the muscle fiber area by the Coulter Counter technique.

Longissimus Dorsi Muscle Fiber Area

Table XLIV gives the A.O.V. for the Longissimus dorsi muscle. Differences in muscle fiber area between sides were not statistically significant (OSL=.5792). The average left side muscle fiber area was 465 microns squared. The average right muscle fiber area was 454 microns squared.

The two methods differed significantly in the Longissimus dorsi (OSL=.0011). The average muscle fiber area by the Coulter Counter technique was 284 microns squared, while that of the Photomicrographic technique was 635 microns squared. A 95% confidence interval for the difference in the methods of muscle fiber area estimation was 351 ± 44 microns squared.

The side x method interaction was not statistically significant (OSL=.3435). Since this interaction was not significant, the muscle fiber area obtained for the Photomicrographic technique parallels the muscle fiber area obtained by the Coulter Counter technique. Figure 10 shows a plot of muscle fiber area ranked by the Coulter Counter

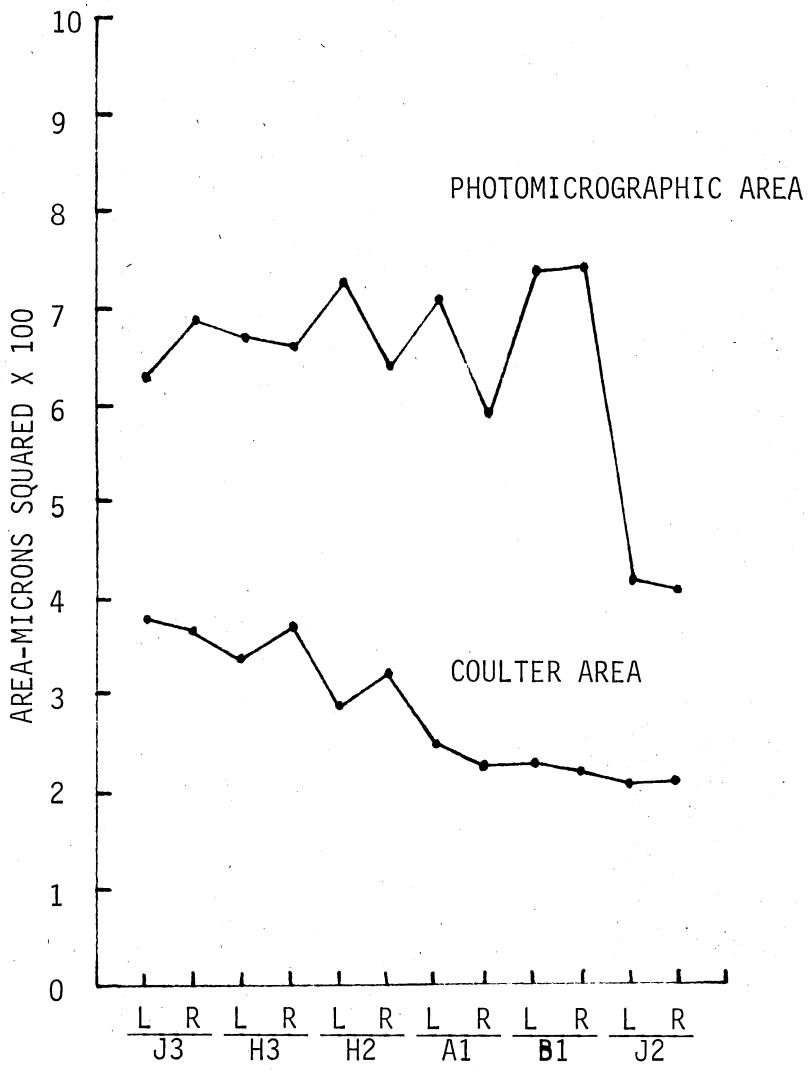


Figure 10. Plot of Muscle Fiber Area by the Coulter Counter and Photomicrographic Techniques in the Longissimus dorsi

technique against muscle fiber area by the Photomicrographic technique. These results suggest that there was less overall variation in the Coulter Counter technique as compared to the Photomicrographic technique, which was verified by the smaller standard deviation of 16 microns squared compared to 48 microns squared, respectively.

Sartorius Muscle Fiber Area

In Table XLV is presented the analysis of variance for muscle fiber area in the Sartorius. Muscle fiber area in the left and right side of the Sartorius was not significantly different (OSL=.5931). The average left side muscle fiber area was 691 microns squared. The average right side muscle fiber area was 707 microns squared.

Methods were statistically significant (OSL=.0028). The average muscle fiber area by the Coulter Counter technique was 447 microns squared. The Photomicrographic technique average muscle fiber area was 951 microns squared. A 95% confidence interval for the difference in the methods for estimating muscle fiber area was 504 ± 187 microns squared.

The side x method interaction was not statistically significant (OSL=.9310). Thus muscle fiber area by the Photomicrographic technique parallels muscle fiber area by the Coulter Counter technique. This conclusion is supported by the graphs in Figure 11.

Semitendinosus Muscle Fiber Area

In Table XLVI is presented the analysis of variance for muscle fiber area in the Semitendinosus. Muscle fiber area in the left and right side of the Semitendinosus was statistically significant

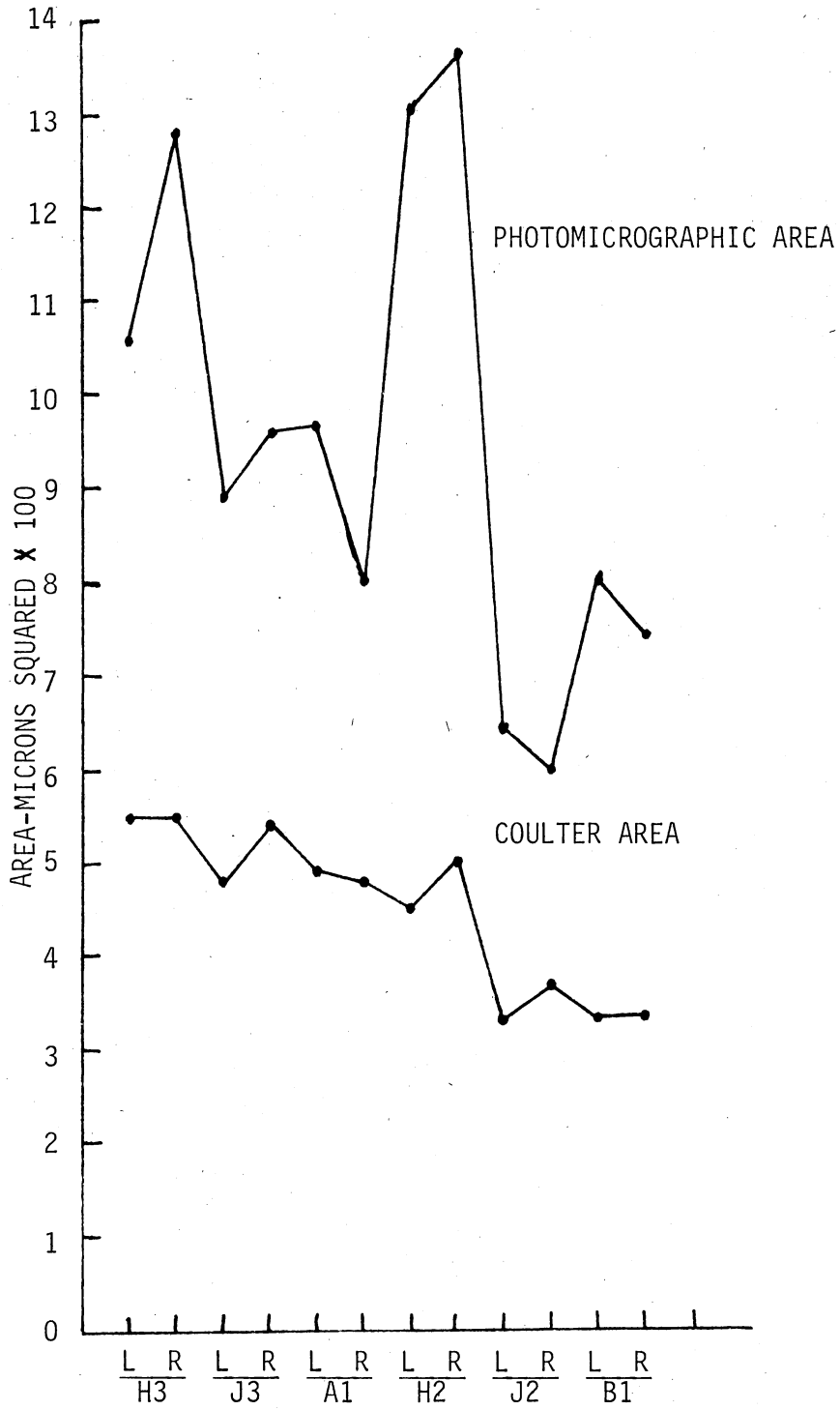


Figure 11. Plot of Muscle Fiber Area by the Coulter Counter and Photomicrographic Techniques in the Sartorius

(OSL=.0195). The mean muscle fiber area in the left side was 605 microns squared. The mean muscle fiber area in the right side was 654 microns squared. A 95% confidence interval of the difference in muscle fiber area between the left and right side of the Semitendinosus was 49 $\pm$ 31 microns squared.

Methods were statistically significant (OSL=.0010). The average muscle fiber area by the Photomicrographic technique was 854 microns squared. Average muscle fiber area by the Coulter Counter technique was 405 microns squared. A 95% confidence interval for the difference in methods for estimating muscle fiber area was 449 $\pm$ 122 microns squared.

The side x method interaction was not statistically significant (OSL=.0922). Figure 12 displays a plot of muscle fiber area ranked by the Coulter Counter technique against Photomicrographic technique. The left side muscle fiber area in the Semitendinosus was always less than the right side muscle fiber area by the Photomicrographic technique. However, the left side muscle fiber area was sometimes greater than the right side muscle fiber area by the Coulter Counter technique.

Triceps Brachii Muscle Fiber Area

In Table XLVII is presented the analysis of variance for muscle fiber area in the Triceps brachii, lateral head. Muscle fiber area in the left and right side of the Triceps brachii was not significantly different (OSL=.9479). The mean muscle fiber area in the left side was 643 microns squared. The mean muscle fiber area in the right side was 641 microns squared. The very close results for the left and

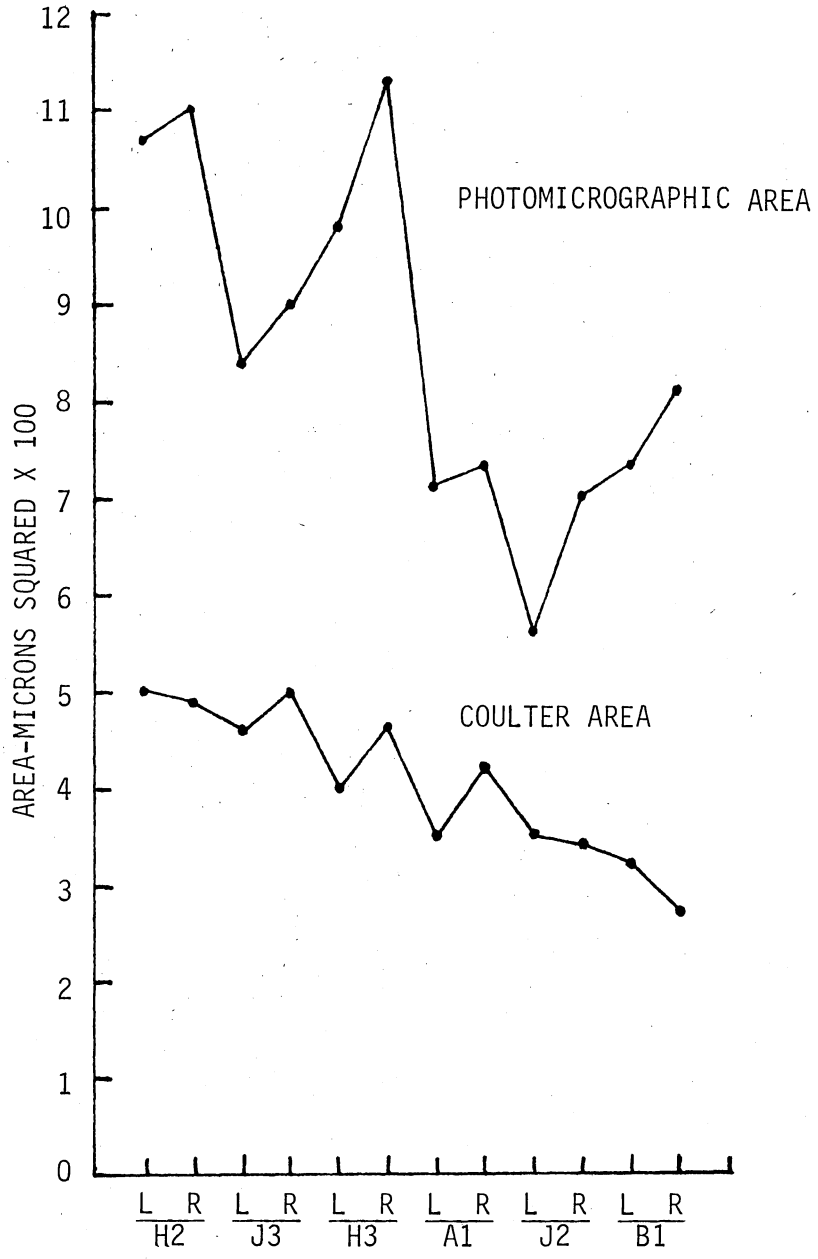


Figure 12. Plot of Muscle Fiber Area by the Coulter Counter and Photomicrographic Techniques in the Semitendinosus

right side of the Triceps brachii indicate a high degree of similarity in muscle fiber area.

Methods were statistically significant (OSL=.0008). The average muscle fiber area by the Coulter Counter technique was 440 microns squared. Average muscle fiber area by the Photomicrographic technique was 844 microns squared. A 95% confidence interval for the difference in the methods for estimating muscle fiber area was 404 $\pm$ 110 microns squared.

The side x method interaction was not statistically significant (OSL=.7105). Muscle fiber area by the Photomicrographic technique parallels muscle fiber area by the Coulter Counter technique since the side x method interaction was nonsignificant. The graphs in Figure 13 tend to confirm this result.

Cumulative Technique Examination

Muscle fiber area was examined using a split-split-plot design to assess the overall relationship between the two techniques (Table XLVIII).

Fiber area in the different muscles was statistically significant (OSL=.0001). The average muscle fiber area in the Longissimus dorsi, Sartorius, Semitendinosus and Triceps brachii were 460, 699, 630 and 642 microns squared, respectively.

Differences between sides were not statistically significant (OSL=.5211). However, the Semitendinosus examined separately did show a statistically significant side effect. The muscle x side interaction of the split-plot was not statistically significant (OSL=.2087).

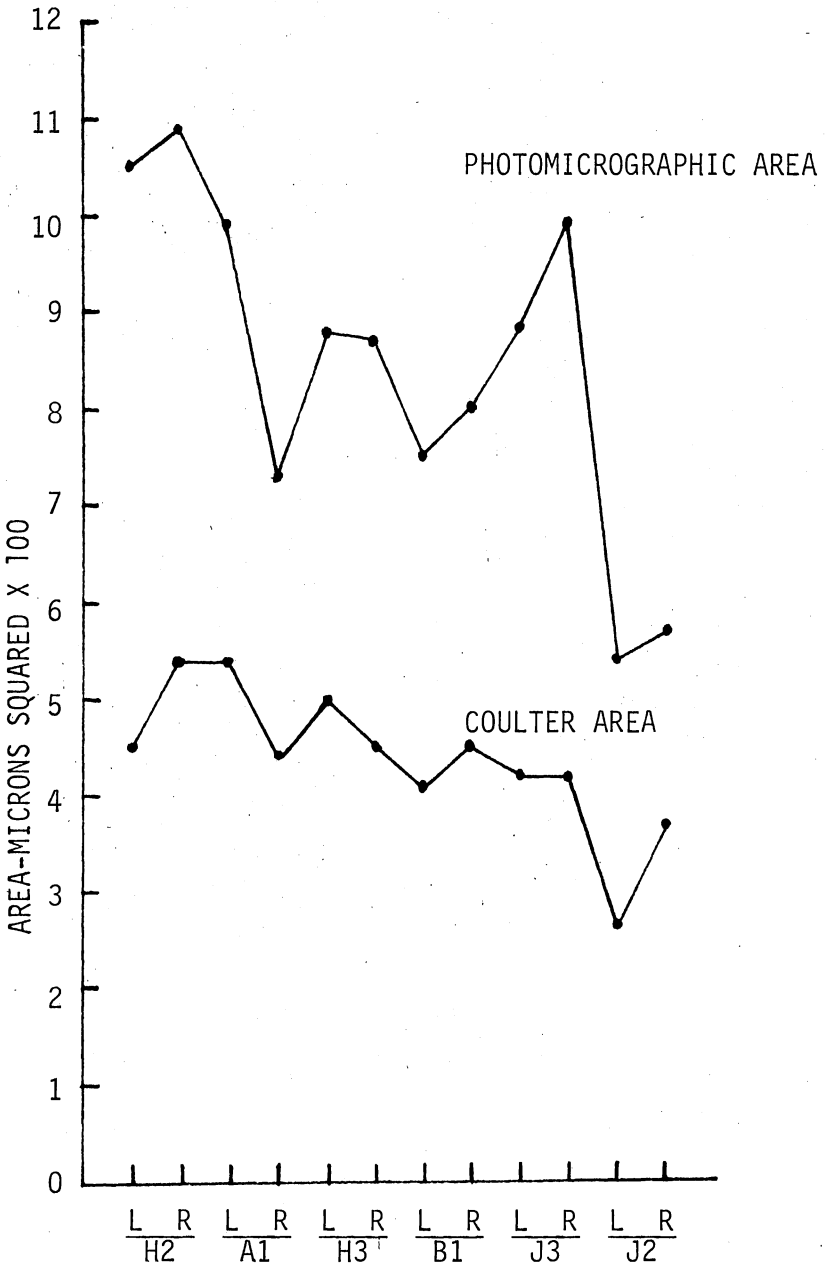


Figure 13. Plot of Muscle Fiber Area by the Coulter Counter and Photomicrographic Techniques in the Triceps brachii

Muscle fiber area was statistically significant between the methods (OSL=.0007). The average muscle fiber area by the Coulter Counter was 394 microns squared. The mean muscle fiber area by the Photomicrographic technique was 821 microns squared. A 95% confidence interval for the difference between the methods was 427 $\pm$ 96 microns squared.

The muscle x method interaction was not statistically significant (OSL=.1325). This suggests that both methods yielded similar results in the muscles studied. The side x method interaction was not statistically significant (OSL=.9341). This interaction indicated the different side x method lines are parallel or related. The muscle x side x method interaction was not statistically significant (OSL=.2559).

Correlations

Simple correlations were not used to assess the relationship between the Coulter Counter technique and the Photomicrographic technique. A cross products analysis was performed and the animal x side interaction used to assess the relationship which exists between the two methods.

In Table XLIX is presented the cross products correlation coefficients of the animal x side interaction for muscle fiber area between the two techniques. A nonsignificant relationship between the two methods ($r=-0.24$; $P > .05$) occurred for the Longissimus dorsi. A nonsignificant relationship between the two methods ($r=-0.12$; $P > .05$) occurred for the Sartorius. In the Semitendinosus, a nonsignificant relationship was obtained between the two methods ($r=-0.45$; $P > .05$).

The Triceps brachii, lateral head was the best muscle for sizing by the Coulter Counter. However, a nonsignificant relationship between the two methods ($r=0.69$; $P > .05$) occurred for the Triceps brachii, lateral head.

Several uncontrollable factors may have affected the relationship between the muscle fiber area by the Coulter Counter and by the Photomicrographic technique.

The Photomicrographic technique has the problems of obtaining the muscle fibers in focus by the microscope. The preparation of the specimen and the eyesight of the operator primarily affect the ability to focus. Muscle fiber area was assumed to be from transverse slices. However, the ability to obtain a transverse slice is difficult for muscle tissue and probably impossible. If nontransverse slices of muscle fibers occurred, then the area obtained was an overestimate of the true muscle fiber area.

The Coulter Counter technique has several uncontrollable sources of error, also. The basic assumption of the nature of particles to be sized by the Coulter Counter is such that, if at all, ionic conduction but not electronic conduction through the particles is possible. All biological cells fall into this category (Katchel, 1976). The Coulter Counter measures and sizes particles according to the resistance-change pulse height produced by the particles. This electrical pulse cannot be directly interpreted as the volume of the particles unless specific conditions for all parameters are satisfied. The most important parameter involved in this article was the resistivity of the muscle fibers. This parameter is involved in the volume calibration of the muscle fibers. If the resistivity of the particles is not

negligible, the measured pulse height is reduced, and a smaller volume is obtained (Katchel, 1976).

Another possible source of error introduced in the Coulter Counter technique was the division of muscle fiber volume by the thickness or length of the tissue slice. The area obtained by this division will only give the correct area if the muscle fibers are symmetrical. This assumption, after viewing many photomicrographs of muscle fibers, is not correct. Therefore, many factors were not controlled in this experiment which affected the relationship between the muscle fiber area by the Coulter Counter and Photomicrographic technique.

Conductivity of Muscle Fibers

The results from this experiment strongly suggest that conductivity of muscle fibers was present and should be considered when sizing muscle fibers by the Coulter Counter procedure. A plot of the difference in techniques against muscle fiber area of the four muscles is presented in Figure 14. The difference in the techniques was small for the smallest muscle fibers and large for the largest muscle fibers. It is believed that the conductivity of the muscle fibers was the source of this result.

Conclusions

No statistically significant relationship was found to exist between the muscle fiber area obtained by the Coulter Counter technique and the Photomicrographic technique. An explanation for the lack of relationship was explained. Results suggested that conductivity of muscle fibers was present and inhibited accurate sizing by the

Coulter Counter. Either of the techniques may be used to size muscle fibers; however, the Coulter Counter technique has a consistently smaller standard error than the photomicrographic technique, and is considerably more rapid and less tedious.

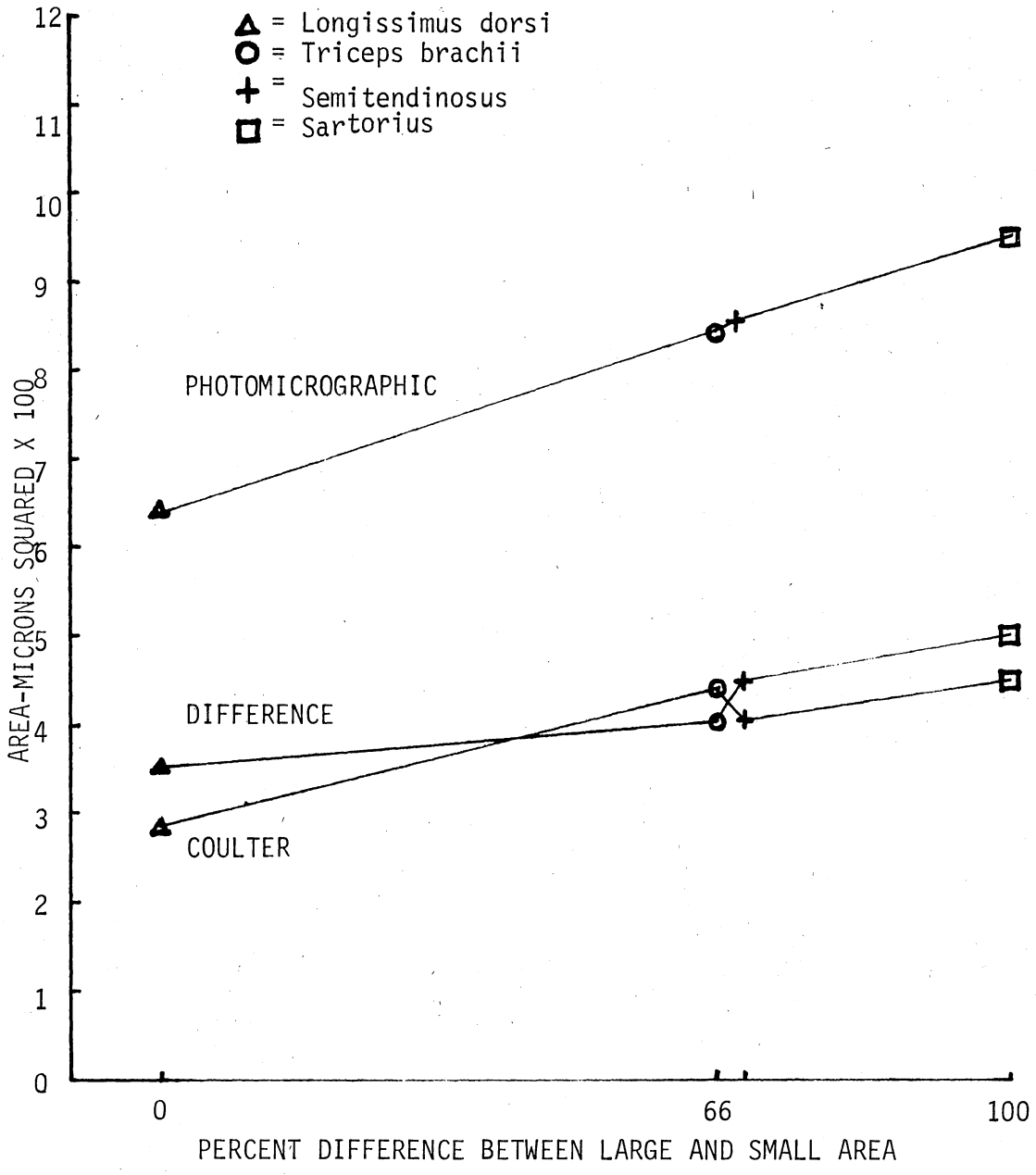


Figure 14. Plot of Photomicrographic and Coulter Counter Muscle Fiber Area and the Difference in Techniques by Muscle

CHAPTER IV

ESTIMATES OF TOTAL NUCLEAR NUMBER AND PROTEIN TO DNA RATIOS

Introduction

The nucleus of a cell is known to be responsible for maintaining and developing the cell. Muscle fibers are the result of fusions between myoblasts early in the development of muscle and are multinucleated cells. Muscle fiber number is determined genetically before birth, and all that can take place after birth is an alteration in size (Stickland et al., 1975). However, total amounts of DNA or nuclear numbers in mammalian muscles continue to increase in postnatal life at slower rates than in the embryo (Young, 1970). The most popular theory as to how muscle fibers gain additional nuclei is via satellite cell incorporation (Burleigh, 1974). Cheek (1968) used the total nuclear number to represent muscle cell population. Several articles have shown animals with larger muscle fiber number have a greater total nuclear number (Ezekwe and Martin, 1975 and Powell and Aberle, 1975).

Protein accumulation exceeds the rate of new nuclear number addition during growth. Therefore, investigators have suggested that the protein to DNA ratio in an organ should be related to the cell size (Winick and Noble, 1965 and Cheek, 1966).

The objectives of this experiment were to determine the relationship between: estimates of total nuclear number and estimates of total muscle fiber number; estimates of the protein to DNA ratio in muscle and estimates of muscle fiber area and sources of variability in the estimates of total nuclear number and estimates of the protein to DNA ratio of muscles.

Materials and Methods

General procedures for obtaining experimental material and processing muscle tissue was the same as that outlined in Chapters II and III of this thesis.

Estimating Total DNA and Nuclear Number

Two (.4-.6) gram samples from each of the three locations were obtained for each muscle. The muscle samples were weighed and the DNA extracted from the muscle tissue by the procedure in Escoubas (1977).

Each one-half gram sample was enlarged to represent the total DNA content of the muscle by the following equation:

$$\text{Total DNA (grams)} = \frac{\text{DNA micrograms}}{\text{100 milligrams}} \times .00001 \times \text{Muscle weight (grams)}$$

The total nuclear number was obtained from total DNA assuming that a normal diploid nucleus contains 6.2 picograms of DNA (Enesco and Leblond, 1962).

$$\text{Total Nuclear Number} = \frac{\text{Total DNA (grams)}}{6.2 \times 10^{-12} \text{ (grams)}}$$

Protein Determination and Protein/DNA

Two one gram samples were taken from each of three locations within a muscle. The samples were prepared for protein determination by the procedure in Escoubas (1977). Total muscle protein in grams was obtained by the following equation:

$$\text{Total muscle protein (grams)} = \frac{\%\text{Protein}}{\text{gram}} \times .01 \times \text{Muscle weight(grams)}$$

The protein per DNA ratio was obtained by the following equation:

$$\text{Protein/DNA} = \frac{\text{Total grams Protein}}{\text{Total grams DNA}}$$

Results and Discussion

Nuclei in the Longissimus Dorsi

The analysis of variance for estimated total nuclear number in the Longissimus dorsi is presented in Table L. The left and right side estimated total nuclear number was not statistically significant (OSL=.5142). The average estimated total nuclear number was 5.496 billion nuclei for the left side and 5.848 billion nuclei for the right side.

Estimated nuclear number from the 25, 50 and 75% locations was not statistically significant (OSL=.7988). Averages for estimated total nuclear number at the 25, 50 and 75% locations were 5.730, 5.550 and 5.735 billion nuclei, respectively. No statistically significant side x location interaction was present in the Longissimus dorsi for estimated total nuclear number (OSL=.8832).

Nuclei in the Sartorius

An analysis of variance for estimated total nuclear number in the Sartorius is presented in Table LI. Left and right side estimated total nuclear number were not statistically significant (OSL=.2364). The mean estimated total nuclear number in the left Sartorius was 571 million nuclei. The average estimated total nuclear number in the right Sartorius was 534 million nuclei.

Estimated nuclear number at the 25, 50 and 75% locations was not statistically significant (OSL=.0853). Averages for estimated total nuclear number at the 25, 50, and 75% locations was 572, 531 and 554 million nuclei, respectively. No statistically significant side x location interaction was present in the Sartorius for estimated total nuclear number (OSL=.5062).

Nuclei in the Semitendinosus

The analysis of variance for estimated total nuclear number in the Semitendinosus is presented in Table LII. Left and right side estimated total nuclear were not statistically significant (OSL=.7217). The average estimated total nuclear number in the left and right Semitendinosus was 2.79 and 2.75 billion nuclei, respectively.

Estimated nuclear number at the 25, 50 and 75% locations was not statistically significant (OSL=.0775). Averages for estimated total nuclear number at the 25, 50 and 75% locations was 2.65, 2.78 and 2.88 billion nuclei, respectively. No statistically significant side x location interaction was present in the Semitendinosus for estimated total nuclear number (OSL=.2129).

Nuclei in the Triceps Brachii

The analysis of variance for estimated total nuclear number in the Triceps brachii, lateral head, is presented in Table LIII. Left and right side estimated total nuclear number was not statistically significant (OSL=.5104). The average estimated total nuclear number was 1.21 billion nuclei for the left side. The average estimated total nuclear number in the right side was 1.25 billion nuclei.

Estimated total nuclear number at the 25, 50 and 75% locations was not statistically significant (OSL=.6435). Averages for estimated total nuclear number at the 25, 50 and 75% locations were 1.23, 1.21 and 1.25 billion nuclei, respectively. The side x location interaction for estimated total nuclear number was not statistically significant (OSL=.5560). The individual observations from which the estimated total nuclear number were taken were sufficiently uniform at the three locations chosen in these experiments. These data would suggest that the location from which the estimate is taken in the Longissimus dorsi, Sartorius, Semitendinosus and Triceps brachii, lateral head yield similar results.

Longissimus Dorsi Protein/DNA

The analysis of variance for protein to DNA ratio in the Longissimus dorsi is presented in Table LIV. The left and right side protein to DNA ratio was not statistically significant (OSL=.3204). The average protein to DNA ratio was 148 for the left side and 153 for the right side.

The protein to DNA ratio estimated at the 25, 50 and 75%

locations were not statistically significant (OSL=.3802). Averages for the protein to DNA ratio at the 25, 50 and 75% locations were 145, 152 and 156, respectively. No statistically significant side x location interaction was present in the Longissimus dorsi for the protein to DNA ratio (OSL=.9697).

Semitendinosus Protein/DNA

The analysis of variance for protein to DNA ratio in the Semitendinosus is presented in Table LV. The left and right side protein to DNA ratio was not statistically significant (OSL=.9884). The average protein to DNA ratio for the left side was 161. The average protein to DNA ratio for the right side was 161.

The protein to DNA ratio estimated at the 25, 50 and 75% locations were statistically significant (OSL=.0416). Averages for the protein to DNA ratio at the 25, 50 and 75% locations were 171, 159 and 153, respectively. The location near the proximal attachment, the 25% location, was larger than the 50 or 75% location estimates. These data suggest that the Semitendinosus is not homogeneous in composition and that location of the estimate will have a significant effect on the results obtained.

No statistically significant side x location interaction was present in the Semitendinosus for the protein to DNA ration (OSL=.5984).

Triceps Brachii, Lateral Head, Protein/DNA

The analysis of variance for protein to DNA ratio in the Triceps brachii, lateral head, is presented in Table LVI. The left and right side protein to DNA ratio was not statistically significant

(OSL=.8601). The average protein to DNA ratio for the left side was 150. The average protein to DNA ratio for the right side was 151.

The protein to DNA ratio estimated at the 25, 50 and 75% locations were not statistically significant (OSL=.8144). Averages for the protein to DNA ratio at the 25, 50 and 75% locations were 150, 152 and 149, respectively. The side x location interaction for protein to DNA was not statistically significant (OSL=.5468).

Correlations of Protein/DNA with Muscle Fiber Area

Many claims have been made in the literature that the protein to DNA ratio of a muscle is a good indicator of cell size (Winick and Noble, 1965 and Cheek, 1968). The only index of cell size of the animals used in this experiment was muscle fiber area. Therefore, muscle fiber area of the animals was used to examine the relationship between the protein to DNA ratio and muscle fiber area.

After muscle fiber number and DNA determinations were made, not enough muscle tissue remained in the Sartorius to perform a protein analysis. Therefore, no protein to DNA ratio relationship with muscle fiber area was obtained in the Sartorius.

In Table LVII are cross product correlations of Coulter Counter muscle fiber area with the protein to DNA ratio. The animal x side interaction of this table was used to assess the statistical relationship between the two variables. The relationship between the protein to DNA ratio and muscle fiber area by the Coulter Counter technique was statistically significant ($r=.90$; $R < .01$). However, the relationship between the two variables in the Semitendinosus and

Triceps brachii, lateral head was not statistically significant ($r=.20$; $P > .05$) and ($r=-0.09$; $P > .05$), respectively.

In Table LXIII are cross product correlations of Photomicrographic muscle fiber area with protein to DNA ratio. The relationship between the protein to DNA ratio and muscle fiber area by the Photomicrographic technique was not statistically significant in the Longissimus dorsi ($r=-0.43$; $P > .05$), Semitendinosus ($r=-0.67$; $P > .05$) and Triceps brachii ($r=0.51$; $P > .05$).

Three correlation coefficients showed a decreasing relationship between the protein to DNA ratio and muscle fiber area. Only one correlation coefficient was statistically significant. These results are different from other reports that the protein to DNA ratio gives an estimate of cell size (Cheek, 1968; Winick and Noble, 1965). These authors observed an increase in the protein to DNA ratio during normal growth in rats. Cell size also increased during normal growth in rats. No attempt was made by the authors to correlate the variables. A more reasonable explanation of the protein to DNA ratio, after observing the results of the present data, would be that the ratio is the expressed genetic potential of the nuclei at a certain point in time. Different animals and breeds attain different sizes due to this genetic potential. Therefore, the protein to DNA ratio in an individual animal with increasing time may be related to cell size; however, a group of animals, at a specific point in time, may exhibit no relationship between protein to DNA ratio and cell size.

Total Nuclear Number and Muscle Fiber Number

Cheek (1968) believed it reasonable to assume that almost all the

DNA in a muscle is in the muscle fibers. This assumption appears to be reasonable since other tissue components of muscle are relatively small compared to muscle fibers. Therefore, total nuclear number and the number of muscle fibers may have some relationship.

Correlations were obtained for total nuclear number and muscle fiber number estimated by the Coulter Counter technique and photomicrographic techniques. In Chapter II both techniques were related in estimating muscle fiber number. A cross product analysis was performed and the animal x side interaction with 5 degrees of freedom was used to assess the level of statistical significance. This variance component also has the variability of the readings added into the component. The animal x side correlation with estimated total nuclear number and muscle fiber number estimated by the Coulter Counter technique is presented in Table LIX. The relationship with nuclear number and muscle fiber number was not statistically significant in any of the muscles examined. In Table LX is the relationship with nuclear number and muscle fiber number estimated by the Photomicrographic technique. The relationship between nuclear number and muscle fiber number was not statistically significant in any of the muscles examined. Several of the correlation coefficients were negative. Decisions involving the relationship between estimated total nuclear number and muscle fiber number are difficult to make. However, these data indicate muscle fiber number and total nuclear number area not related in calves 15 days of age.

Conclusions

Estimation of total DNA or nuclear number in the four muscles, from samples taken at the three locations along the muscle, were not significantly different. Therefore, samples taken from any of the three locations chosen in this experiment will satisfactorily estimate total nuclear number.

Estimation of the protein to DNA ratio was significantly affected by sampling locations in the Semitendinosus. Sampling location estimates of protein to DNA ratio in the Longissimus dorsi and Triceps brachii, lateral head, did not significantly affect the protein to DNA ratio.

The protein to DNA ratio relationship with muscle fiber area was not statistically significant in calves 15 days of age. However, the correlation of muscle fiber area by the Coulter Counter Technique and protein to DNA ratio in the Longissimus dorsi was statistically significant. Protein to DNA ratios should be considered the expressed genetic potential of nuclei at a given point in time.

Muscle fiber number and estimated total nuclear number showed no statistically significant relationship in any of the four muscles examined in this experiment.

CHAPTER V

A PRINCIPLE FOR OBTAINING TRUE MUSCLE FIBER AREA

Introduction

Microscopic estimation of muscle fiber cross-sectional area or diameter has been a popular technique to investigate changes of muscle fibers during the growth process (Swanson et al., 1965; Livingston et al., 1966; Crystal et al., 1969; Miller et al., 1975). Investigations which use cross-sectional area or diameter attempt to align fibers to obtain a tissue slice perpendicular to the longitudinal axis of a muscle fiber. Other techniques involve separating the muscle fibers and measuring the projection diameter or width of the muscle fibers.

Muscle fibers not sliced perpendicular to the longitudinal axis of the muscle fibers will result in an apparent cross-sectional area or diameter which is always greater than the true cross-sectional area or diameter. Measurement of the apparent cross-sectional area gives incorrect results. Indeed, muscle fibers are not true cylinders but appear in cross-section to be primarily irregular polygons (Eisenberg et al., 1974; Swatland, 1975). Therefore, the projection diameter method gives incorrect results.

The actual diameter of a muscle fiber is related to the apparent

diameter times the cosine of the angle θ (Maxwell et al., 1974). The angle θ is the angular displacement from a true perpendicular to the longitudinal axis of a muscle fiber. For accurate results to be obtained, fibers must be cylinders.

The common problem encountered in research involving muscle fiber area determination is the angle θ. Angle θ is not known for the experimental material. Although almost all investigators of fiber area or diameter will eventually conclude that true cross-sections perpendicular to the longitudinal axis of muscle fibers are difficult to obtain in research.

Swatland (1975) measured the maximum endomyseal sheath width parallel to the mean diameter axis. The arc sine of the ratio, minimum endomyseal sheath width to the maximum endomyseal sheath width, gives the angle θ. This method of accessing the angle θ is tedious and highly dependent on the operator's judgement.

Determining the angle θ easily and accurately might be valuable in future studies of muscle fiber area or diameter during the growth process. Therefore, the objectives of this research was the development of a technique to determine the angle θ.

Materials and Methods

From a 15 day old Holstein calf, the lateral head of the Triceps brachii was removed then wrapped in one thickness of heavy duty aluminum foil then frozen pre-rigor by immersion in liquid nitrogen. The muscle was sectioned perpendicular to the longitudinal axis with a bandsaw and four one-quarter inch cores were taken parallel with the longitudinal axis. The length of the cores were adjusted to be less

than or equal to the diameter of the core. The core was then placed in Isoton, a phosphate buffered saline solution, at 24°C and allowed to enter thaw rigor. After thaw rigor, the core was positioned in O.C.T. compound on a chilled microtome chuck and refrozen with Cryo-kwik.

Tissue slices 60 microns in length were taken from each core and transferred with a wire loop to a vial of Isoton. Manual adjustment resulted in lengths less than or greater than 60 microns for each slice. Slices were then disrupted into small fiber rods by a Heat Systems Inc. Sonifier Cell disruptor at 70 watts output for 15 seconds. This technique dislodges a sufficient number of muscle fibers from which a random sample may be taken.

A pasteur pipette was used to transfer the small fiber rods in suspension to a microscope slide. Photomicrographs of fibers were taken with a 35 mm camera using Kodak Tri-X film. The developed negative of the fiber rods were projected and traced on paper. The angle θ was measured with a compass on tracings of the muscle fibers. One side of the rod was assumed parallel to the longitudinal axis and the angle θ was measured for both ends. Since the side which faces up on the rod could be either side, a coin was flipped to select the angle which faces up on a microscope slide.

Results

The angle θ was observed to vary within each core (Figure 15). The number of fibers in each core were not sufficient to make any judgement about the distribution of angle θ; therfore, the observations for each core were pooled. A histogram of the pooled cores versus

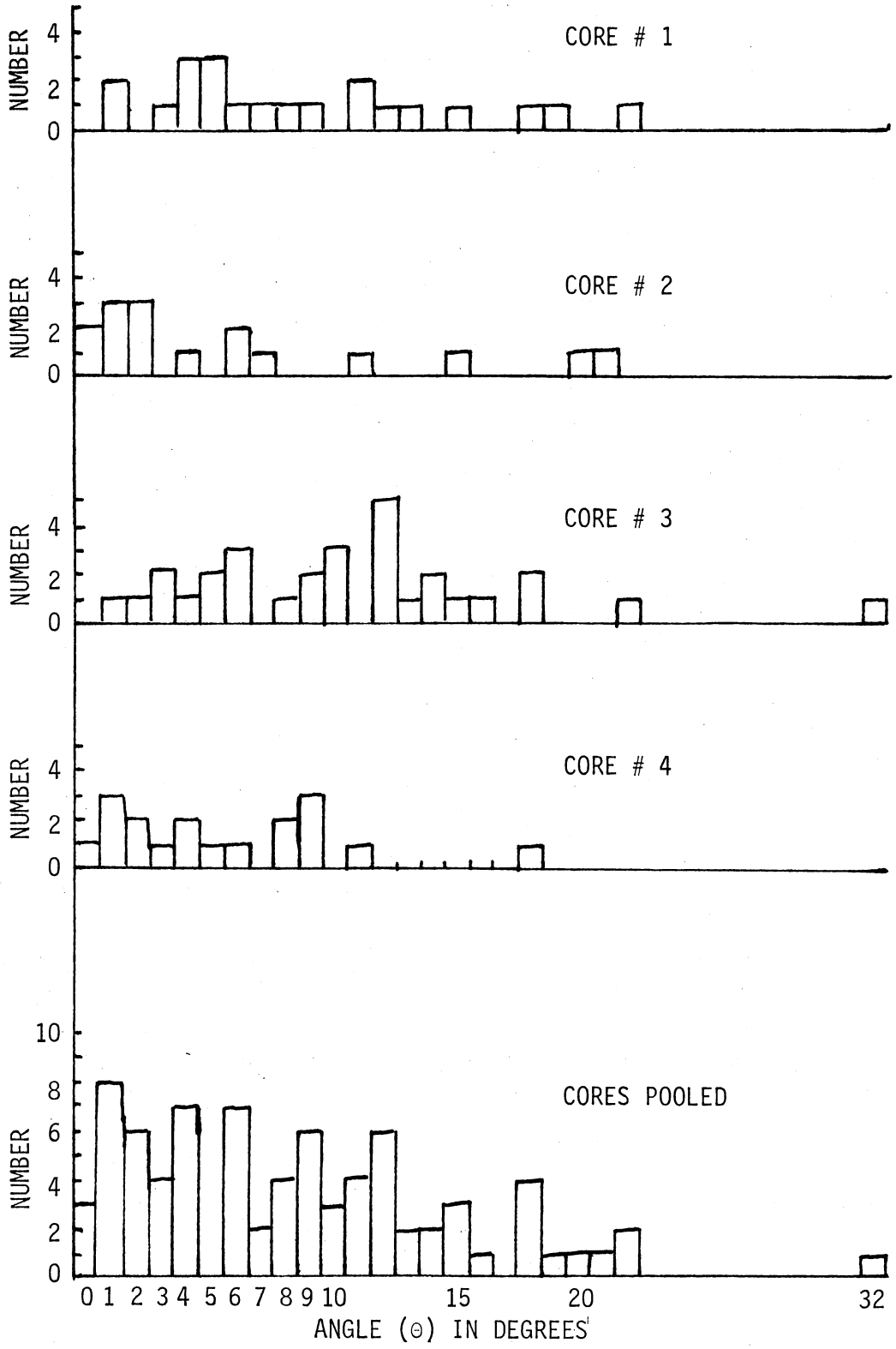


Figure 15. Histogram of the Number of Observations and Angle (Θ)

angle θ appeared to be uniformly distributed from 0 to 15 degrees. A Chi-Square Goodness of fit test was used in testing the hypothesis that angle θ was uniformly distributed on the interval 0 to 15 degrees. The test was not significant at α=.005.

Since the angle θ can be estimated for tissue slices, a theory was modified to utilize this information. A very long cylindrical body has its longitudinal axis lying in the plane, x, y, of a three-dimensional co-ordinate system, x, y, z and is inclined at an angle θ to the x-axis (Figure 16). An infinitely small slice of thickness, dx, cut parallel to the y, z, plane will have a volume:

$$dv = A(x,\theta)\, dx \qquad \text{(Equation (1))}$$

where $A(x,\theta)$ is the cross-sectional area of the body for any value of x and θ. The body has total volume:

$$v = \int_0^{G(\theta)} A(x,\theta)\, dx = G(\theta)\cdot A(\theta) \qquad \text{(Equation (2))}$$

where $A(\theta)$ is the average cross-sectional area obtained by sectioning the body in one given direction θ, and $G(\theta)$ is the projection length of the body on the x-axis at this inclination.

If all angles of inclination between ψ and ϕ occur with equal probability, the mean cross-sectional area, $\bar{a}$, becomes:

$$\bar{a} = \frac{1}{\psi-\phi}\int_\phi^\psi A(\theta)\, d\theta = \frac{v}{\theta}\int_\phi^\psi \frac{d\theta}{G(\theta)} \qquad \text{(Equation (3))}$$

The above is a standard derivation of Stereology for the cross-sectional area, a specific derivation for ϕ=0 and $\psi=\frac{\pi}{2}$ was reported by Weibel and Gomez (1962). Stereology uses small bodies and considers all possible orientations of a body. However, muscle fibers are large bodies which can be oriented to a certain degree.

Consider the projection length $G(\theta)$.

$$G(\theta) = L\cdot\cos\theta + D\cdot\sin\theta \qquad \text{(Equation (4))}$$

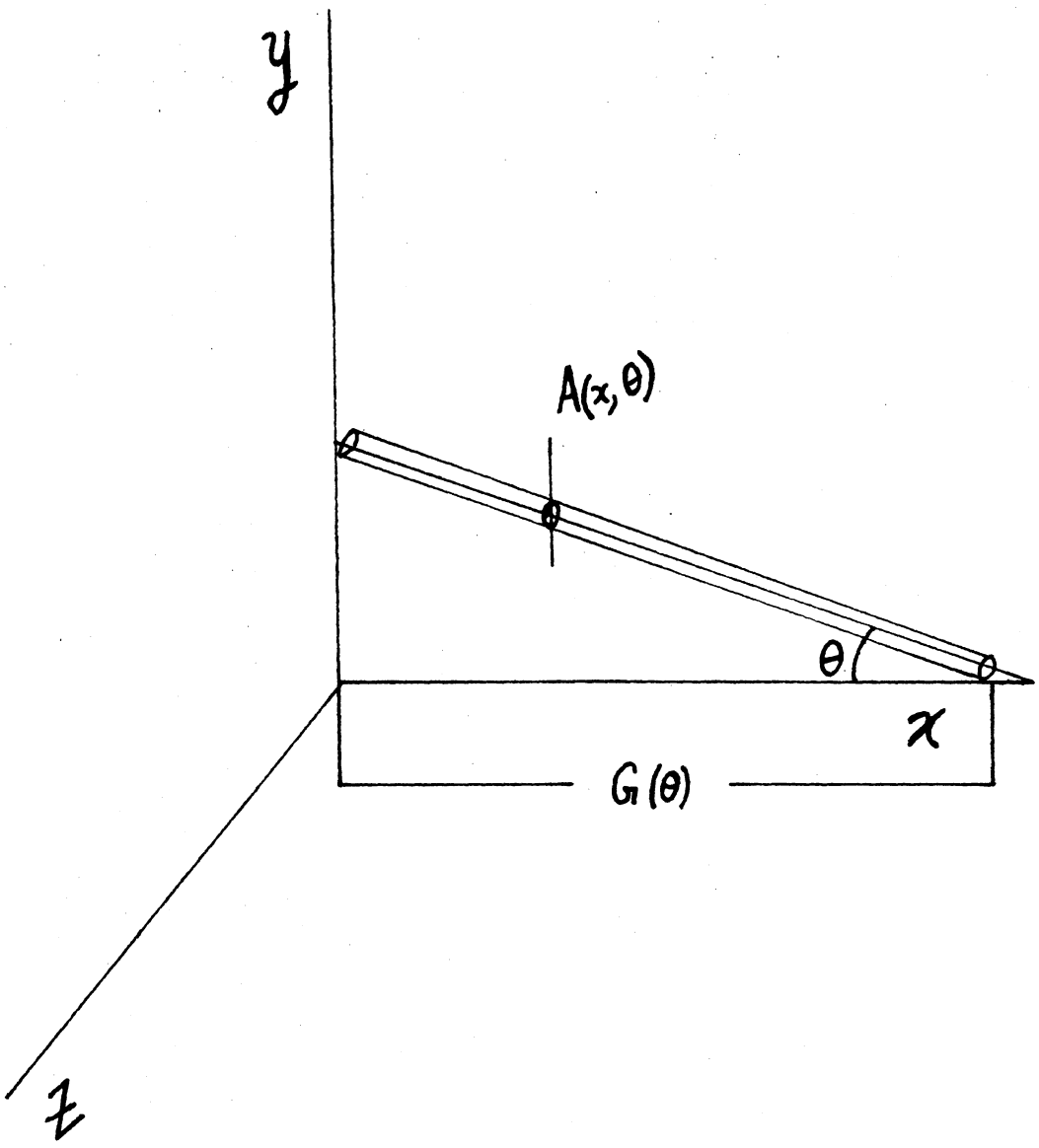


Figure 16. A Long Cylindrical Muscle Fiber inclined at Angle (θ) with the X-Axis, Sectioned Parallel to the (y,z) Plane

and substitute the projection length, equation 4, into equation 3. In order to complete the integration in a simpler way a new value

$$K = \frac{D}{L} \qquad \text{(Equation (5))}$$

is defined.

Integration of the above.

$$\bar{a} = \frac{V}{\psi-\phi} \int_{\phi}^{\psi} \frac{d\theta}{L\cdot\cos\theta + D\cdot\sin\theta} \qquad \text{(Equation (6))}$$

Equation 6 is a rational function of sine and cosine, therefore, the following substituations can be made:

$$u = \tan\frac{\theta}{2} \quad \text{if } -\pi < \theta < \pi$$

$$d\theta = \frac{2du}{1 + u^2}$$

$$\sin\theta = \frac{2u}{1 + u^2}$$

$$\cos\theta = \frac{1 - u^2}{1 + u^2}$$

into equation 6.

$$\bar{a} = \frac{V}{\psi-\phi} \int_{\tan\frac{\phi}{2}}^{\tan\frac{\psi}{2}} \frac{\frac{2}{1+u^2}\,du}{L\cdot\left(\frac{1-u^2}{1+u^2}\right) + D\cdot\left(\frac{2u}{1+u^2}\right)} \qquad \text{(Equation (7))}$$

Simplifying the above.

$$\bar{a} = \frac{V}{\psi-\phi} \int_{\tan\frac{\phi}{2}}^{\tan\frac{\psi}{2}} \frac{2du}{L - Lu^2 + 2Du} \qquad \text{(Equation (8))}$$

Completing the square in the denominator.

$$L - Lu^2 + 2KLu \qquad \text{(Equation (9))}$$

$$-L\,(u^2 - 2Ku) + L \qquad \text{(Equation (10))}$$

$$-L\,(u^2 - 2Ku + K^2) + L + LK^2 \qquad \text{(Equation (11))}$$

$$-L\,(u + K)^2 + L\,(1 + K^2) \qquad \text{(Equation (12))}$$

Using the result of equation 12, equation 8 becomes

$$\bar{a} = \frac{v}{\psi-\phi} \int_{\tan\frac{\phi}{2}}^{\tan\frac{\psi}{2}} \frac{2du}{L\left[(1 + K^2) - (u + K)^2\right]} \qquad \text{(Equation (13))}$$

Rearranging the denominator

$$\bar{a} = \frac{v}{(\psi-\phi)L} \int_{\tan\frac{\phi}{2}}^{\tan\frac{\psi}{2}} \frac{2du}{(\sqrt{1 + K^2})^2 - (u + K)^2} \qquad \text{(Equation (14))}$$

Using a table of Integrals, equation 14 becomes

$$\bar{a} = \left(\frac{A}{\psi-\phi}\right)\left(\frac{2}{2\sqrt{1 + K^2}}\right) \ln \frac{\sqrt{1 + K^2} + (u + K)}{\sqrt{1 + K^2} - (u + K)} \Bigg|_{\tan\frac{\phi}{2}}^{\tan\frac{\psi}{2}} \qquad \text{(Equation (15))}$$

Completing the integration.

$$\bar{a} = \left(\frac{A}{(\psi-\phi)\sqrt{1 + K^2}}\right) \ln \frac{\left[\sqrt{1 + K^2} + (\tan\frac{\psi}{2} + K)\right]\left[\sqrt{1 + K^2} - (\tan\frac{\phi}{2} + K)\right]}{\left[\sqrt{1 + K^2} - (\tan\frac{\psi}{2} + K)\right]\left[\sqrt{1 + K^2} + (\tan\frac{\phi}{2} + K)\right]} \qquad \text{(Eq.(16))}$$

Muscle fibers of striated muscle attain great lengths (Maxwell et al., 1974; Goldspink, 1962) while muscle fiber diameter is restricted to a smaller maximum value generally less than 100 microns in diameter. A reasonable assumption for most studies would be to assume K is zero. Equation 16 then becomes:

$$\bar{a} = A\left(\frac{1}{(\psi-\phi)} \ln \frac{(1 + \tan\frac{\psi}{2})(1 - \tan\frac{\phi}{2})}{(1 - \tan\frac{\psi}{2})(1 + \tan\frac{\phi}{2})}\right) \qquad \text{(Equation (17))}$$

where $\bar{a}$ is the mean cross-sectional area of the muscle fiber sliced at all angles from ϕ to ψ. "A" is the true cross-sectional area of the muscle fiber and the quantity in brackets now designated as CF is the correction factor which utilized the upper and lower limit of the

angle θ which the fiber deviates from a perpendicular to the longitudinal axis of the muscle fibers.

Equation 17 is derived for one muscle fiber; however, the equation can be logically extended to an infinite number of muscle fibers. Where $\bar{a}$ is the mean of the mean cross-sectional area of the muscle fibers sliced at all angles from ϕ to ψ. "A" is the mean true cross-sectional area of the muscle fibers and the correction factor, CF, is the same as for a single fiber.

The correction factor, CF, like the mean of the true cross-sectional area is dependent on the sample size. Equation 17 also assumes muscle fiber area to be uniform over a small interval of the muscle fiber length.

Application

Correction factors for muscle fiber area sliced between a fixed lower limit of zero degrees and variable upper limit for angle θ are shown in Table LXI. As the upper limit increases the correction factor, CF, increases (Figure 17). The actual mean area is increased only 1.16% when angle θ was uniformly distributed between 0 and 15 degrees.

The data obtained from this experiment were from cores which had gone through thaw rigor. Fresh muscle tissue or tissue from post-rigor muscle which is taken and aligned then sectioned could react differently. However, the angle θ will probably still be uniformly distributed over some fixed interval. If the above statement is true then considerable error could be present in most microscopic determinations of muscle fiber area.

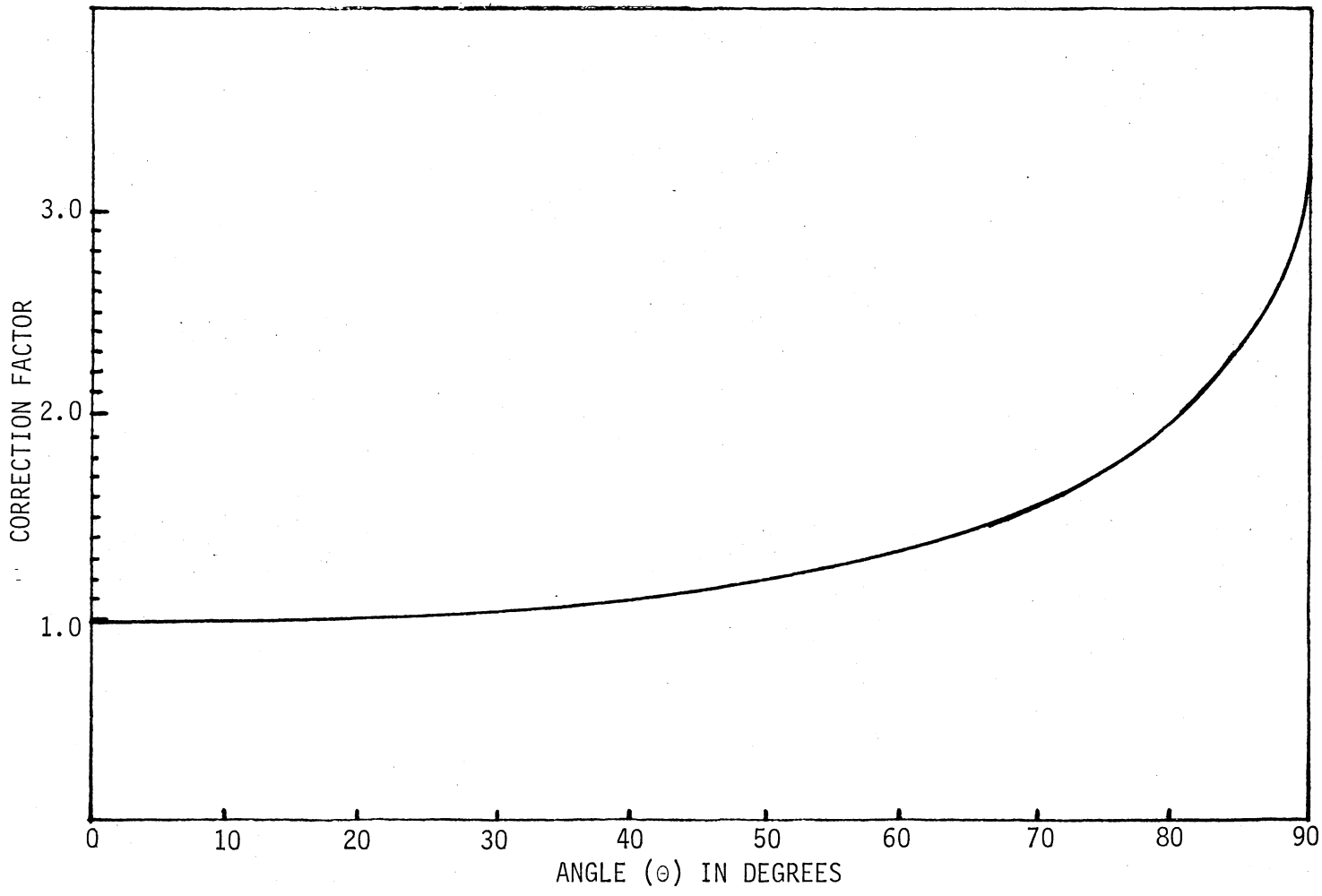


Figure 17. Correction Factor versus Angle (Θ)

From the theory aligning the muscle fibers is obviated. The correct mean area of a muscle fiber distribution can be obtained by the equation derived and a knowledge of angle θ. Obviously, the equation will become useful in experimental studies on the Longissimus dorsi. The muscle fibers are known not to run parallel with the longitudinal axis of the Longissimus dorsi. Studies by microscope of muscle fiber number at certain locations will be based on tissue slices taken parallel to the cross-sectional area of the muscle and the fiber area from these slices can be corrected with the equation derived.

The equation derived will be useful in obtaining accurate fiber area. Relationships of muscle fiber area with other body parameters during growth may need re-examination, since the error that results in fiber area measurements is not random but a fixed factor depending on how the fibers were sliced.

Summary

A technique was developed for measuring the angle which muscle fibers are sliced from a true perpendicular to the longitudinal axis. Angles from a true perpendicular to the longitudinal axis appear to be uniformly distributed between 0 and 15 degrees in thaw rigor tissue which was aligned to obtain transverse sections. An equation of stereology was modified for use with muscle fibers using the information of how the angle was distributed to obtain true fiber area.

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APPENDIX

TABLES

TABLE I

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER FIELD IN THE SEMITENDINOSUS BY THE MICROSCOPIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	7,307,041.33	
Side	1	2,745,633.33	.0922
Animal x Side	1	54,945.33	
Split Plot Analysis			
Location	2	11,925.06	.7434
Side x Location	2	29,841.65	.5100
'A x L + A x S x L'[a]	4	37,044.15	
Split Split Plot Analysis			
End	1	110,208.33	.0707
Side x End	1	1,408.33	.8076
Location x End	2	37,676.02	.2733
Side x Loc x End	2	32,154.02	.3207
'A x E + A x E x S/L'[b]	6	23,225.54	
READ (Ani Side Loc End)	24	14,014.08	
Total	47	7,307,041.33	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE II

ANALYSIS OF VARIANCE FOR TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SEMITENDINOSUS BY THE MICROSCOPIC TECHNIQUE.

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	397,701,707,152	
Side	1	556,634,000	.9373
Animal x Side	1	57,072,135,668	
Split Plot Analysis			
Location	2	29,788,973,312	.3723
Side x Location	2	21,386,929,562	.5289
'A x L + A x S x L'[a]	4	24,839,550,971	
Split Split Plot Analysis			
End	1	151,901,006,463	.0471
Side x End	1	2,764,825,184	.7469
Location x End	2	8,589,468,231	.7236
Side x Loc x End	2	27,437,520,593	.3919
'A x E + A x E x S/L'[b]	6	24,839,550,971	
READ (Ani Side Loc End)	24	14,917,393,819	
Total	47	29,456,907,683	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE III

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER FIELD IN THE SARTORIUS BY THE MICROSCOPIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	3,183,215.02	
Side	1	1,489,313.02	.3672
Animal x Side	1	613,590.19	
Split Plot Analysis			
Location	2	42,825.27	.4386
Side x Location	2	93,550.02	.2225
'A x L + A x S x L'[a]	4	41,761.23	
Split Split Plot Analysis			
End	1	147,741.02	.0993
Side x End	1	99,099.19	.1623
Location x End	2	43,660.02	.3910
Side x Loc x End	2	17,951.69	.6578
'A x E + A x E x S/L'[b]	6	39,417.94	
READ (Ani Side Loc End)	24	8,532.35	
Total	47	139,090.67	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE IV

ANALYSIS OF VARIANCE FOR TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SARTORIUS BY THE MICROSCOPIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	261,289,063,647	
Side	1	38,808,837,804	.4483
Animal x Side	1	27,718,540,655	
Split Plot Analysis			
Location	2	89,210,639,022	.1777
Side x Location	2	42,556,042,064	.3655
'A x L + A x S x L'[a]	4	32,465,691,301	
Split Split Plot Analysis			
End	1	17,180,821,184	.0688
Side x End	1	4,048,234,133	.3282
Location x End	2	10,343,204,279	.1307
Side x Loc x End	2	5,500,005,740	.2874
'A x E + A x E x S/L'[b]	6	3,556,968,992	
READ (Ani Side Loc End)	24	994,904,949	
Total	47	17,432,927,182	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE V

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER FIELD IN THE TRICEPS BRACHII LATERAL HEAD BY THE MICROSCOPIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	3,520,291.69	
Side	1	7,072,513.02	.2775
Animal x Side	1	1,445,255.02	
Split Plot Analysis			
Location	2	112,677.33	.7099
Side x Location	2	258,386.08	.5107
'A x L + A x S x L'[a]	4	298,844.29	
Split Split Plot Analysis			
End	1	30,351.02	.6659
Side x End	1	27,888.52	.6782
Location x End	2	76,601.33	.6195
Side x Loc x End	2	57,103.58	.6951
'A x E + A x E x S/L'[b]	6	145,756.65	
READ (Ani Side Loc End)	24	7,193.90	
Total	47	326,561.84	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE VI

ANALYSIS OF VARIANCE FOR TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE TRICEPS BRACHII LATERAL HEAD BY THE MICROSCOPIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	231,210,324,918	
Side	1	864,910,115,678	.0264
Animal x Side	1	1,542,765,490	
Split Plot Analysis			
Location	2	124,823,106,979	.1651
Side x Location	2	226,429,633,030	.0759
'A x L + A x S x L'[a]	4	42,655,004,692	
Split Split Plot Analysis			
End	1	3,341,820,002	.7946
Side x End	1	5,023,996,727	.7532
Location x End	2	9,805,952,767	.8204
Side x Loc x End	2	4,246,668,671	.9156
'A x E + A x E x S/L'[b]	6	47,773,206,662	
READ (Ani Side Loc End)	24	3,099,615,475	
Total	47	50,389,144,167	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE VII

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER FIELD IN THE LONGISSIMUS DORSI BY THE MICROSCOPIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	10,792,033.3	
Side	1	732,108.0	.5033
Animal x Side	1	748,001.3	
Split Plot Analysis			
Location	2	444,787.1	.5150
Side x Location	2	530,213.8	.5366
'A x L + A x S x L'[a]	4	561,787.0	
Split Split Plot Analysis			
End	1	33,496.3	.7614
Side x End	1	134,408.3	.5590
Location x End	2	544,610.9	.2799
Side x Loc x End	2	558,438.9	.2726
'A x E + A x E x S/L'[b]	6	343,342.3	
READ (Ani Side Loc End)	24	47,545.1	
Total	47	469,030.5	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE VIII

ANALYSIS OF VARIANCE FOR TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE LONGISSIMUS DORSI BY THE MICROSCOPIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	1	1.5493758 E+11[a]	
Side	1	5.4821783 E+10	.8592
Animal x Side	1	1.2007761 E+12	
Split Plot Analysis			
Location	2	2.1116234 E+12	.0432
Side x Location	2	2.7037603 E+11	.5526
'A x L + A x S x L'[b]	4	2.7154790 E+11	
Split Split Plot Analysis			
End	1	2.7382620 E+10	.6950
Side x End	1	7.8085021 E+10	.5176
Location x End	2	3.8559372 E+11	.1724
Side x Loc x End	2	3.4605348 E+11	.1983
'A x E + A x E x S/L'[c]	6	1.6155621 E+11	
READ (Ani Side Loc End)	24	2.6111177 E+10	
Total	47	2.2181901 E+11	

[a] E=exponent of 10.

[b] Animal x Location + Animal x Side x Location.

[c] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE IX

A SPLIT SPLIT PLOT EXPECTED MEAN SQUARES TABLE
FOR THE MICROSCOPE TECHNIQUE

Animal	$\sigma^2 + 24\sigma^2_A$
Side	$\sigma^2 + 12\sigma^2_{AS} + 24\sigma^2_S$
Animal x Side	$\sigma^2 + 12\sigma^2_{AS}$
Location	$\sigma^2 + 4\sigma^2_{e2} + 16\sigma^2_L$
Side x Location	$\sigma^2 + 4\sigma^2_{e2} + 8\sigma^2_{SL}$
'A x L + A x S x L'	$\sigma^2 + 4\sigma^2_{e2}$
End	$\sigma^2 + 2\sigma^2_{e3} + 24\sigma^2_E$
Side x End	$\sigma^2 + 2\sigma^2_{e3} + 12\sigma^2_{SE}$
Location x End	$\sigma^2 + 2\sigma^2_{e3} + 4\sigma^2_{SLE} + 8\sigma^2_{LE}$
Side x Location x End	$\sigma^2 + 2\sigma^2_{e3} + 4\sigma^2_{SLE}$
'A x E + A x E x S/L'	$\sigma^2 + 2\sigma^2_{e3}$
Read (ASLE)	σ^2

TABLE X

SELECTED OUTPUT SETTINGS AND TIME EFFECT ON TISSUE SLICE DISRUPTION

	Slice #1	Slice #2	Slice #3
Core #1	Output #2 1 minute Some clumps	Output #3 1 minute Homogenized cells	Output #4 45 seconds Some clumps
Core #2	Output #2 1 minute Disrupted	Output #2 2 minutes Homogenized cells	Output #3 1 minute Cell breakage
Core #3	Output #4 15 seconds Disrupted	Output #5 10 seconds Disrupted	Output #3 1 minute Some clumps
Core #4	Output #4 15 seconds Disrupted	Output #4 10 seconds Disrupted	Output #4 5 seconds Some clumps
Core #5	Output #5 5 seconds Disrupted	Output #5 5 seconds Disrupted	Output #5 5 seconds Disrupted

TABLE XI

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE LONGISSIMUS DORSI BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	582,753,141	
Side	1	3,189,248,986	.2023
Animal x Side	5	1,490,512,725	
Split Plot Analysis			
Location	2	3,414,661,634	.0216
Side x Location	2	2,074,089,445	.0819
'A x L + A x S x L'[a]	20	735,374,298	
Split Split Plot Analysis			
End	1	263,315,291	.6238
Side x End	1	14,965,212	.9010
Location x End	2	177,999,752	.8441
Side x Loc x End	2	553,275,074	.5976
'A x E + A x E x S/L'[b]	30	1,038,274,553	
READ (Ani Side Loc End)	72	180,702,285	
Total	143	595,385,998	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XII

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE LONGISSIMUS DORSI BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	6.5460573 E+12[a]	
Side	1	1.1705219 E+12	.5739
Animal x Side	5	1.5355302 E+12	
Split Plot Analysis			
Location	2	4.2416704 E+12	.0141
Side x Location	2	1.5276527 E+12	.1734
'A x L + A x S x L'[b]	20	8.0174876 E+11	
Split Split Plot Analysis			
End	1	5.6483322 E+10	.7918
Side x End	1	5.1233909 E+10	.8009
Location x End	2	4.6556897 E+11	.9455
Side x Loc x End	2	3.5165534 E+11	.6643
'A x E + A x E x S/L'[c]	30	8.3145326 E+11	
READ (Ani Side Loc End)	72	1.2760588 E+11	
Total	143	7.2858352 E+11	

[a] E=exponent of 10.

[b] Animal x Location + Animal x Side x Location.

[c] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XIII

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE SARTORIUS BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	7,446,911,629	
Side	1	167,904,960	.3420
Animal x Side	5	151,467,484	
Split Plot Analysis			
Location	2	47,977,939	.6786
Side x Location	2	51,643,719	.6591
'A x L + A x S x L'[a]	20	119,054,142	
Split Split Plot Analysis			
End	1	1,355,329,425	.0038
Side x End	1	951,395,425	.0122
Location x End	2	47,778,475	.7100
Side x Loc x End	2	333,701,450	.1001
'A x E + A x E x S/L'[b]	30	135,217,177	
READ (Ani Side Loc End)	72	91,230,502	
Total	143	380,663,796	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XIV

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SARTORIUS BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	141,482,540,167	
Side	1	61,234,232	.9450
Animal x Side	5	12,307,509,741	
Split Plot Analysis			
Location	2	49,177,985,698	.0382
Side x Location	2	8,876,423,667	.5167
'A x L + A x S x L'[a]	20	12,844,022,673	
Split Split Plot Analysis			
End	1	35,107,758,538	.0752
Side x End	1	69,320,131,768	.0150
Location x End	2	8,999,137,358	.5595
Side x Loc x End	2	20,049,228,018	.1663
'A x E + A x E x S/L'[b]	30	10,581,688,365	
READ (Ani Side Loc End)	72	7,514,630,307	
Total	143	15,126,079,791	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XV

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE SEMITENDINOSUS BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	3,498,583,083	
Side	1	206,196,923	.5061
Animal x Side	5	371,145,224	
Split Plot Analysis			
Location	2	1,618,050,400	.0278
Side x Location	2	358,204,462	.5928
'A x L + A x S x L'[a]	30	378,532,634	
Split Split Plot Analysis			
End	1	114,442,017	.5385
Side x End	1	79,131,726	.6087
Location x End	2	1,267,782,987	.0201
Side x Loc x End	2	104,437,457	.7020
'A x E + A x E x S/L'[b]	30	285,936,657	
READ (Ani Side Loc End)	72	133,671,466	
Total	143	365,164,061	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XVI

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SEMITENDINOSUS BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	2.9120520 E+12[a]	
Side	1	8.0030306 E+11	.1087
Animal x Side	5	2.1244958 E+11	
Split Plot Analysis			
Location	2	6.0738238 E+11	.1360
Side x Location	2	3.7094838 E+11	.2838
'A x L + A x S x L'[b]	20	2.7667392 E+11	
Split Split Plot Analysis			
End	1	1.0807544 E+11	.5175
Side x End	1	4.5347707 E+10	.6480
Location x End	2	1.0710319 E+12	.0117
Side x Loc x End	2	5.8374541 E+10	.7601
'A x E + A x E x S/L'[c]	30	2.0727386 E+11	
READ (Ani Side Loc End)	72	8.7006203 E+10	
Total	143	2.7138356 E+11	

[a] E=exponent of 10.

[b] Animal x Location + Animal x Side x Location.

[c] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XVII

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE TRICEPS BRACHII LATERAL HEAD BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	1,661,048,072	
Side	1	359,711,311	.5247
Animal x Side	5	592,080,192	
Split Plot Analysis			
Location	2	9,828,900	.9780
Side x Location	2	252,919,508	.5652
'A x L + A x S x L'[a]	20	424,479,941	
Split Split Plot Analysis			
End	1	153,325,650	.5973
Side x End	1	24,543,555	.8239
Location x End	2	234,890,742	.6461
Side x Loc x End	2	331,710,971	.5398
'A x E + A x E x S/L'[b]	30	519,862,808	
READ (Ani Side Loc End)	72	113,417,719	
Total	143	319,674,692	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XVIII

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE TRICEPS BRACHII LATERAL HEAD BY THE PHOTOMICROGRAPHIC TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	9.1737440 E+11[a]	
Side	1	9.1737803 E+10	.5798
Animal x Side	5	2.5674981 E+11	
Split Plot Analysis			
Location	2	4.5951563 E+12	.0001
Side x Location	2	4.9896588 E+10	.6613
'A x L + A x S x L'[b]	20	1.1597727 E+11	
Split Split Plot Analysis			
End	1	5.7975401 E+08	.9501
Side x End	1	1.1173728 E+09	.9302
Location x End	2	1.8726468 E+11	.3097
Side x Loc x End	2	1.8126529 E+11	.3213
'A x E + A x E x S/L'[c]	30	1.5358350 E+11	
READ (Ani Side Loc End)	72	3.7929796 E+10	
Total	143	1.7936516 E+11	

[a]E=exponent of 10.

[b]Animal x Location + Animal x Side x Location.

[c]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XIX

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE LONGISSIMUS DORSI BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	4,020,817,664	
Side	1	1,854,390,281	.2624
Animal x Side	5	1,164,880,399	
Split Plot Analysis			
Location	2	2,709,741,226	.1847
Side x Location	2	1,285,260,115	.5622
'A x L + A x S x L'[a]	20	1,479,348,504	
Split Split Plot Analysis			
End	1	10,242,848	.9436
Side x End	1	496,653,333	.6380
Location x End	2	1,291,511,626	.5571
Side x Loc x End	2	425,233,433	.8219
'A x E + A x E x S/L'[b]	30	2,132,971,583	
Slice (Ani Side Loc End)	72	122,592,976	
READ (Ani Side Loc End Slice)	288	2,485,168	
Total	431	331,396,310	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XX

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE LONGISSIMUS DORSI BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	8.7793380 E+12	
Side	1	9.4510993 E+11	.5474
Animal x Side	5	1.3995303 E+12	
Split Plot Analysis			
Location	2	1.3474350 E+13	.0015
Side x Location	2	1.6136699 E+12	.3392
'A x L + A x S x L'[a]	20	1.4103061 E+12	
Split Split Plot Analysis			
End	1	1.8647315 E+10	.9126
Side x End	1	2.9208813 E+11	.6804
Location x End	2	1.4846319 E+12	.5787
Side x Loc x End	2	2.3786630 E+11	.8669
'A x E + A x E x S/L'[b]	30	1.6555155 E+12	
Slice (Ani Side Loc End)	72	8.7060741 E+10	
READ (Ani Side Loc End Slice)	288	1.8484969 E+09	
Total	431	3.9546068 E+11	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XXI

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE SARTORIUS BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	9,716,963,046	
Side	1	271,573,959	.5269
Animal x Side	5	442,516,677	
Split Plot Analysis			
Location	2	281,117,893	.3795
Side x Location	2	278,969,159	.3823
'A x L + A x S x L'[a]	20	275,026,221	
Split Split Plot Analysis			
End	1	682,319,737	.1117
Side x End	1	36,633,426	.7108
Location x End	2	176,979,493	.5174
Side x Loc x End	2	729,161,959	.0745
'A x E + A x E x S/L'[b]	30	259,424,344	
Slice (Ani Side Loc End)	72	77,851,670	
READ(Ani Side Loc End Slice)	288	1,595,478	
Total	431	171,852,565	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XXII

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SARTORIUS BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	881,403,019,031	
Side	1	65,328,157,490	.2926
Animal x Side	5	47,206,538,897	
Split Plot Analysis			
Location	2	142,228,442,418	.0513
Side x Location	2	39,504,499,772	.5949
'A x L + A x S x L'[a]	20	41,497,736,085	
Split Split Plot Analysis			
End	1	12,093,309,782	.5687
Side x End	1	2,938,432,771	.6955
Location x End	2	39,765,954,449	.1332
Side x Loc x End	2	44,871,700,619	.1045
'A x E + A x E x S/L'[b]	30	18,553,920,663	
Slice (Ani Side Loc End)	72	5,905,423,993	
READ (Ani Side Loc End Slice)	288	124,202,061	
Total	431	16,481,860,666	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XXIII

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE SEMITENDINOSUS BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	11,770,657,080	
Side	1	305,424,300	.5348
Animal x Side	5	674,164,082	
Split Plot Analysis			
Location	2	1,600,108,886	.1406
Side x Location	2	119,250,803	.8531
'A x L + A x S x L'[a]	20	742,620,352	
Split Split Plot Analysis			
End	1	72,848,981	.6047
Side x End	1	26,561,793	.7486
Location x End	2	969,399,684	.0338
Side x Loc x End	2	120,688,090	.6355
'A x E + A x E x S/L'[b]	30	257,285,385	
Slice (Ani Side Loc End)	72	59,217,081	
READ (Ani Side Loc End Slice)	288	1,102,169	
Total	431	221,345,381	

[a]Animal x Location + Animal x Side x Location.

[b]Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XXIV

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SEMITENDINOSUS BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	5.8248689 E+12[a]	
Side	1	1.2335693 E+12	.2012
Animal x Side	5	5.7294804 E+11	
Split Plot Analysis			
Location	2	5.0618653 E+11	.3527
Side x Location	2	3.9923728 E+10	.9166
'A x L + A x S x L'[b]	20	4.5945539 E+11	
Split Split Plot Analysis			
End	1	1.0422654 E+11	.5230
Side x End	1	3.5246011 E+10	.6770
Location x End	2	6.4408973 E+11	.0493
Side x Loc x End	2	4.3963081 E+10	.8018
'A x E + A x E x S/L'[c]	30	1.9502397 E+11	
Slice (Ani Side Loc End)	72	4.1320955 E+10	
READ (Ani Side Loc End Slice)	288	8.6790202 E+08	
TOTAL	431	1.2551124 E+11	

[a] E=exponent of 10.

[b] Animal x Location + Animal x Side x Location.

[c] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XXV

ANALYSIS OF VARIANCE FOR MUSCLE FIBER COUNT PER SLICE IN THE TRICEPS BRACHII LATERAL HEAD BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	4,033,607,677	
Side	1	89,726,237	.7535
Animal x Side	5	842,304,657	
Split Plot Analysis			
Location	2	113,837,901	.8306
Side x Location	2	532,830,956	.5681
'A x L + A x S x L'[a]	20	603,302,261	
Split Split Plot Analysis			
End	1	29,557,870	.8149
Side x End	1	40,431,170	.7864
Location x End	2	169,277,545	.7464
Side x Loc x End	2	725,221,090	.2900
'A x E + A x E x S/L'[b]	30	562,588,856	
Slice (Ani Side Loc End)	72	44,298,559	
READ (Ani Side Loc End Slice)	288	1,935,271	
Total	431	139,935,446	

[a] Animal x Location + Animal x Side x Location.

[b] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XXVI

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE TRICEPS BRACHII LATERAL HEAD BY THE COULTER COUNTER TECHNIQUE

Source of Variation	df	Mean Square	Observed Significance Level
Whole Plot Analysis			
Animal	5	7.5904955 E+11[a]	
Side	1	3.1175982 E+09	.9260
Animal x Side	5	3.5183814 E+11	
Split Plot Analysis			
Location	2	7.7619477 E+12	.0001
Side x Location	2	1.9919722 E+11	.5850
'A x L + A x S x L'[b]	20	2.1513768 E+11	
Split Split Plot Analysis			
End	1	3.5383097 E+10	.6820
Side x End	1	4.8442764 E+10	.6338
Location x End	2	1.7916285 E+11	.5733
Side x Loc x End	2	1.6758882 E+11	.5492
'A x E + A x E x S/L'[c]	30	2.0278284 E+11	
Slice (Ani Side Loc End)	72	1.0734271 E+10	
READ (Ani Side Loc End Slice)	288	6.6748254 E+08	
Total	431	7.7977996 E+10	

[a] E=exponent of 10.

[b] Animal x Location + Animal x Side x Location.

[c] Animal x End + Animal x Side x End + Animal x Location x End + Animal x Side x Location x End.

TABLE XXVII

A SPLIT SPLIT PLOT EXPECTED MEAN SQUARES TABLE FOR THE CROSS PRODUCTS ANALYSIS OF VARIANCE

Animal	$\sigma^2 + 8\sigma^2_A$
Side	$\sigma^2 + 6\sigma^2_{e1} + 36\sigma^2_S$
Animal x Side	$\sigma^2 + 6\sigma^2_{e1}$
Location	$\sigma^2 + 2\sigma^2_{e2} + 24\sigma^2_L$
Side x Location	$\sigma^2 + 2\sigma^2_{e2} + 12\sigma^2_{SL}$
'A x L + A x S x L'	$\sigma^2 + 2\sigma^2_{e2}$
End	$\sigma^2 + \sigma^2_{e3} + 36\sigma^2_E$
Side x End	$\sigma^2 + \sigma^2_{e3} + 18\sigma^2_{SE}$
Location x End	$\sigma^2 + \sigma^2_{e3} + 2\sigma^2_{SLE} + 12\sigma^2_{LE}$
Side x Location x End	$\sigma^2 + \sigma^2_{e3} + 2\sigma^2_{SLE}$
'A x E + A x E x S/L'	$\sigma^2 + \sigma^2_{e3}$
Read (ASLE)	σ^2

TABLE XXVIII

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER COUNT BY PHOTOMICROGRAPHIC COUNT PER TISSUE SLICE IN THE LONGISSIMUS DORSI

Source	df	Correlation Coefficient
Ani	5	0.834
Side	1	1.000
Ani x Side	5	0.497
'A x L + A x S x L'	20	0.812
End	1	1.000
'A x E + A x E x S/L'	30	0.864
Corrected Total	71	0.768

TABLE XXIX

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER COUNT BY PHOTOMICROGRAPHIC COUNT PER TISSUE SLICE IN THE SARTORIUS

Source	df	Correlation Coefficient
Ani	5	0.889
Side	1	-1.000
Ani x Side	5	-0.106
'A x L + A x S x L'	20	0.533
End	1	1.000
'A x E + A x E x S/L'	30	0.267
Corrected Total	71	0.764

TABLE XXX

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER COUNT BY PHOTOMICROGRAPHIC COUNT PER TISSUE SLICE IN THE SEMITENDINOSUS

Source	df	Correlation Coefficient
Ani	5	0.908
Side	1	1.000
Ani x Side	5	0.408
'A x L + A x S x L'	20	0.822
End	1	1.000
'A x E + A x E x S/L'	30	0.528
Corrected Total	71	0.788

TABLE XXXI

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER COUNT BY PHOTOMICROGRAPHIC COUNT PER TISSUE SLICE IN THE TRICEPS BRACHII, LATERAL HEAD

Source	df	Correlation Coefficient
Ani	5	0.919
Side	1	-1.000
Ani x Side	5	-0.315
'A x L + A x S x L'	20	0.531
End	1	1.000
'A x E + A x E x S/L'	30	0.237
Corrected Total	71	0.437

TABLE XXXII

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER BY PHOTOMICROGRAPHIC ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE LONGISSIMUS DORSI

Source	df	Correlation Coefficient
Ani	5	0.942
Side	1	1.000
Ani x Side	5	0.660
'A x L + A x S x L'	20	0.885
End	1	1.000
'A x E + A x E x S/L'	30	0.868
Corrected Total	71	0.874

TABLE XXXIII

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER BY PHOTOMICROGRAPHIC ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SARTORIUS

Source	df	Correlation Coefficient
Ani	5	0.946
Side	1	-1.000
Ani x Side	5	0.447
'A x L + A x S x L'	20	0.741
End	1	1.000
'A x E + A x E x S/L'	30	0.195
Corrected Total	71	0.752

TABLE XXXIV

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER BY PHOTOMICROGRAPHIC ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE SEMITENDINOSUS

Source	df	Correlation Coefficient
Ani	5	0.888
Side	1	1.000
Ani x Side	5	0.295
'A x L + A x S x L'	20	0.801
End	1	1.000
'A x E + A x E x S/L'	30	0.570
Corrected Total	71	0.777

TABLE XXXV

CORRELATIONS FOR SELECTED VARIANCE COMPONENTS FROM THE CROSS PRODUCTS ANALYSIS FOR COULTER BY PHOTOMICROGRAPHIC ESTIMATED TOTAL MUSCLE FIBER COUNT PER CROSS-SECTIONAL AREA IN THE TRICEPS BRACHII, LATERAL HEAD

Source	df	Correlation Coefficient
Ani	5	0.970
Side	1	-1.000
Ani x Side	5	0.271
'A x L + A x S x L'	20	0.586
End	1	1.000
'A x E + A x E x S/L'	30	0.384
Corrected Total	71	0.750

TABLE XXXVI

ESTIMATES OF MUSCLE FIBER NUMBER AT THREE LOCATIONS BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE LONGISSIMUS DORSI

		Coulter Counter		Photomicrographic	
Ani	Loc	Left	Right	Left	Right
A1	25	1.335±0.135[a]	1.368±0.335	2.099±0.165	1.599±0.596
	50	1.807±0.197	1.902±0.373	2.587±0.246	2.269±0.314
	75	1.575±0.743	1.740±0.407	1.955±0.744	1.795±0.605
B1	25	1.684±0.219	1.738±0.092	3.028±0.192	2.529±0.669
	50	1.784±0.273	1.938±1.242	2.403±0.321	2.398±1.337
	75	2.511±1.094	2.364±0.169	3.459±1.238	3.060±0.627
H2	25	1.729±0.224	1.629±0.199	2.316±0.191	2.386±0.185
	50	1.878±0.141	2.674±0.140	2.379±0.416	4.099±0.994
	75	1.843±0.222	1.704±0.264	2.429±0.206	2.888±0.290
H3	25	1.934±0.374	1.702±0.147	2.571±0.438	2.120±0.115
	50	3.018±0.778	1.883±0.183	3.521±0.692	2.608±0.198
	75	3.126±0.118	2.694±0.145	4.131±0.302	3.438±0.434
J2	25	0.989±0.152	1.393±0.365	1.433±0.172	1.584±0.502
	50	1.233±0.058	1.130±0.197	1.502±0.243	1.857±0.127
	75	2.129±0.720	1.334±0.120	2.322±1.275	1.745±0.253
J3	25	1.419±0.102	1.208±0.267	1.985±0.158	1.446±0.180
	50	1.629±0.331	2.196±0.180	2.049±0.576	2.527±0.792
	75	2.472±0.711	1.810±0.403	3.085±0.615	1.659±0.330

a = Mean ± Standard deviation x 10^6

TABLE XXXVII

ESTIMATES OF MUSCLE FIBER NUMBER AT THREE LOCATIONS BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE SARTORIUS

Ani	Loc	Coulter Counter		Photomicrographic	
		Left	Right	Left	Right
A1	25	2.55±0.70[a]	2.34±0.61	4.07±0.57	4.79±1.04
	50	3.85±0.15	3.68±0.92	5.34±0.60	5.02±0.83
	75	3.98±1.01	2.78±1.36	4.78±0.65	4.69±0.34
B1	25	4.53±0.41	4.97±0.71	5.72±0.68	6.31±0.75
	50	5.86±0.26	6.97±0.40	7.20±0.25	7.27±1.00
	75	5.22±0.82	5.34±0.53	6.64±1.28	6.19±0.38
H2	25	4.18±0.96	3.14±0.51	5.28±0.52	3.64±0.98
	50	4.35±0.47	4.13±0.84	5.35±1.15	5.48±1.04
	75	3.77±0.60	3.26±0.44	5.09±0.97	4.68±1.20
H3	25	6.15±0.09	5.98±0.20	5.78±0.94	7.22±1.61
	50	5.39±0.81	6.64±0.36	6.06±0.27	7.11±1.65
	75	6.14±0.32	5.31±0.54	6.45±1.72	6.22±0.78
J2	25	4.08±0.46	3.66±0.33	5.37±1.29	6.31±0.38
	50	4.47±0.77	3.34±0.24	5.97±1.26	5.77±0.77
	75	3.54±0.72	2.59±0.04	5.13±0.56	4.23±0.60
J3	25	4.91±0.21	4.27±0.18	6.17±0.74	5.24±0.74
	50	3.80±0.09	3.68±0.71	5.17±0.95	5.37±0.64
	75	3.36±0.49	3.63±0.54	4.63±1.83	4.89±0.95

a = Mean ± Standard deviation x 10^5

TABLE XXXVIII

ESTIMATES OF MUSCLE FIBER NUMBER AT THREE LOCATIONS BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE SEMITENDINOSUS

Ani	Loc	Coulter Counter		Photomicrographic	
		Left	Right	Left	Right
A1	25	1.524±0.125[a]	1.545±0.084	1.771±0.339	1.880±0.362
	50	1.250±0.072	1.651±0.314	1.605±0.111	2.128±0.334
	75	1.192±0.109	1.180±0.048	1.520±0.417	1.888±0.230
B1	25	1.522±0.233	2.154±0.142	2.204±0.469	2.094±0.639
	50	1.695±0.347	2.056±0.166	2.297±0.512	2.160±0.345
	75	1.631±0.248	1.711±0.277	2.181±0.177	2.244±0.641
H2	25	1.395±0.136	1.550±0.060	1.819±0.160	1.797±0.229
	50	1.392±0.168	1.647±0.184	1.533±0.099	1.890±0.403
	75	1.481±0.160	1.788±0.379	1.772±0.236	2.738±0.822
H3	25	1.623±0.112	1.515±0.178	2.140±0.129	2.267±0.197
	50	1.715±0.048	1.274±0.055	2.335±0.639	1.779±0.135
	75	1.678±0.322	1.934±0.058	2.226±0.449	2.926±0.180
J2	25	1.288±0.123	1.602±1.184	1.515±0.122	1.862±0.266
	50	1.196±0.098	1.027±0.064	1.391±0.186	1.101±0.204
	75	1.273±0.176	1.394±0.066	1.377±0.160	1.472±0.249
J3	25	1.253±0.128	1.003±0.050	1.668±0.164	1.586±0.192
	50	0.823±0.260	0.827±0.110	1.156±0.466	1.314±0.347
	75	0.988±0.174	0.986±0.090	1.557±0.717	1.445±0.229

a = Mean ± Standard deviation x 10^6

TABLE XXXIX

ESTIMATES OF MUSCLE FIBER NUMBER AT THREE LOCATIONS BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE TRICEPS BRACHII

		Coulter Counter		Photomicrographic	
Ani	Loc	Left	Right	Left	Right
A1	25	0.895±0.042[a]	0.901±0.043	1.086±0.129	1.287±0.103
	50	1.086±0.128	0.857±0.042	1.018±0.135	1.546±0.235
	75	0.549±0.157	0.605±0.181	0.513±0.130	0.910±0.373
B1	25	1.019±0.354	1.204±0.103	1.426±0.698	1.643±0.348
	50	0.968±0.027	1.230±0.248	1.429±0.098	1.723±0.237
	75	0.688±0.087	0.811±0.102	0.974±0.081	0.955±0.161
H2	25	0.941±0.074	1.115±0.335	1.164±0.206	1.141±0.667
	50	0.712±0.184	1.044±0.053	1.035±0.186	1.266±0.261
	75	0.561±0.048	0.505±0.090	0.827±0.218	0.723±0.214
H3	25	0.941±0.132	0.988±0.125	1.327±0.286	1.186±0.113
	50	1.320±0.244	0.963±0.355	1.840±0.437	1.631±0.198
	75	0.672±0.051	0.576±0.046	0.996±0.103	1.021±0.147
J2	25	0.631±0.083	0.651±0.109	1.096±0.158	0.799±0.164
	50	1.103±0.180	0.814±0.124	1.343±0.099	0.966±0.276
	75	0.463±0.083	0.546±0.103	0.558±0.072	0.589±0.119
J3	25	0.928±0.132	0.666±0.115	0.819±0.580	0.960±0.179
	50	1.181±0.076	0.918±0.180	1.601±0.218	1.192±0.317
	75	0.353±0.087	0.521±0.067	0.386±0.046	0.805±0.114

a = Mean ± Standard deviation x 10^6

TABLE XL

ESTIMATES OF FIBER SIZE BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE LONGISSIMUS DORSI

Ani	Side	Coulter Counter			Photomicrographic	
		Volume μ^3	Area μ^2	Diameter μ	Area μ^2	Diameter μ
A1	L	4936.0[a]	246.0	17.0	707.5	29.2
		2528.1[b]	126.4	4.9	315.9	6.9
	R	4669.0	233.0	16.0	586.7	26.6
		2518.8	125.9	5.0	269.5	6.4
B1	L	4628.0	231.0	16.0	742.1	29.6
		2478.1	123.9	4.9	379.0	8.3
	R	4332.0	216.0	15.0	745.7	29.7
		2489.3	124.4	4.9	392.1	7.9
H2	L	5848.0	292.0	18.0	729.6	29.5
		2727.2	136.4	5.7	337.6	7.6
	R	6505.0	325.0	19.0	636.6	27.6
		2879.2	143.9	5.8	326.7	7.0
H3	L	6700.0	335.0	19.0	666.5	28.5
		2828.5	199.9	6.2	267.7	6.3
	R	7410.0	370.0	20.0	661.5	28.4
		2880.2	203.6	6.1	258.8	5.8
J2	L	4184.0	209.0	15.0	424.5	22.5
		2237.8	111.8	4.5	205.8	5.8
	R	4203.0	210.0	15.0	404.5	21.8
		2275.9	113.8	4.6	218.5	6.1
J3	L	7590.0	379.0	21.0	627.5	27.6
		3101.2	219.2	6.4	278.2	6.2
	R	7300.0	365.0	20.0	692.1	29.0
		2991.9	211.5	6.3	292.0	6.2

a = Mean.
b = Standard deviation.

TABLE XLI

ESTIMATES OF FIBER SIZE BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE SARTORIUS

Ani	Side	Coulter Counter			Photomicrographic	
		Volume μ^3	Area μ^2	Diameter μ	Area μ^2	Diameter μ
A1	L	9816.0[a]	490.0	24.0	968.6	34.4
		2607.4[b]	219.0	5.7	372.3	7.2
	R	9653.0	482.0	23.0	803.5	31.3
		3436.1	242.8	6.8	313.5	6.5
B1	L	6737.0	336.0	19.0	806.4	31.2
		3034.5	151.7	6.3	358.6	7.4
	R	6714.0	335.0	19.0	735.7	29.9
		2895.9	144.8	6.0	283.6	6.4
H2	L	9332.0	466.0	23.0	1293.2	40.1
		2840.6	142.2	6.5	377.2	5.9
	R	10002.0	500.0	24.0	1363.6	41.0
		3192.1	159.6	7.1	453.6	7.2
H3	L	10989.0	549.0	25.0	1064.7	36.1
		3012.1	253.0	6.5	378.5	7.1
	R	10111.0	505.0	24.0	1281.8	39.5
		2933.9	246.5	6.5	491.9	8.2
J2	L	6555.0	327.0	19.0	640.9	27.9
		2391.4	119.6	5.4	261.1	5.7
	R	7463.0	373.0	20.0	602.9	26.4
		2700.0	135.0	5.8	430.9	8.3
J3	L	9582.0	479.0	23.0	892.5	33.2
		2743.4	230.5	6.1	270.7	5.7
	R	10874.0	543.0	25.0	958.6	34.3
		3185.8	267.6	6.8	343.3	6.3

a = Mean.
b = Standard deviation.

TABLE XLII

ESTIMATES OF FIBER SIZE BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE SEMITENDINOSUS

Ani	Side	Coulter Counter			Photomicrographic	
		Volume μ^3	Area μ^2	Diameter μ	Area μ^2	Diameter μ
A1	L	6991.0[a]	349.0	20.0	708.4	29.4
		2835.8[b]	200.4	6.1	288.1	6.2
	R	8389.0	419.0	22.0	728.8	29.8
		3022.5	213.6	6.3	279.5	5.9
B1	L	6441.0	322.0	19.0	726.7	29.6
		2831.2	141.5	5.7	321.4	6.8
	R	5424.0	271.0	17.0	808.3	31.1
		2483.3	124.2	5.4	383.7	7.9
H2	L	10015.0	500.0	24.0	1072.6	36.3
		3146.6	157.3	6.9	357.6	6.9
	R	9890.0	494.0	24.0	1097.8	36.8
		2946.4	147.3	6.6	363.7	6.5
H3	L	7958.0	397.0	21.0	983.2	34.8
		2671.5	224.4	6.4	353.2	6.5
	R	9310.0	465.0	23.0	1127.1	37.4
		3072.3	258.1	6.9	349.5	5.9
J2	L	7049.0	352.0	20.0	558.7	26.1
		2410.7	120.5	5.3	209.4	5.6
	R	6876.0	343.0	20.0	701.5	28.9
		2560.4	128.0	5.7	331.5	7.7
J3	L	9193.0	459.0	23.0	835.6	31.8
		3223.3	227.8	6.5	357.4	7.3
	R	9891.0	494.0	24.0	904.2	32.8
		3269.6	231.1	6.4	426.9	8.5

a=Mean.
b=Standard deviation.

TABLE XLIII

ESTIMATES OF FIBER SIZE BY PHOTOMICROGRAPHIC AND COULTER COUNTER TECHNIQUES IN THE TRICEPS BRACHII, LATERAL HEAD

Ani	Side	Coulter Counter Volume μ^3	Coulter Counter Area μ^2	Coulter Counter Diameter μ	Photomicrographic Area μ^2	Photomicrographic Diameter μ
A1	L	10722.0[a]	536.0	25.0	990.8	35.1
		3390.6[b]	246.7	6.7	317.7	5.6
	R	8756.0	437.0	22.0	732.9	29.7
		2932.4	213.3	6.1	327.5	7.2
B1	L	8286.0	414.0	22.0	753.4	30.4
		3064.7	153.2	6.5	292.4	6.1
	R	8944.0	447.0	23.0	792.9	31.2
		3025.9	151.3	6.1	338.0	6.2
H2	L	9909.0	495.0	24.0	1045.0	35.7
		2961.8	148.1	6.5	447.5	7.3
	R	10804.0	540.0	25.0	1085.2	36.6
		3319.9	165.9	7.1	383.6	6.4
H3	L	10064.0	503.0	24.0	882.5	33.2
		3354.5	237.1	6.6	262.2	4.8
	R	9067.0	453.0	23.0	867.0	32.6
		3144.7	222.2	6.3	333.1	6.5
J2	L	5177.0	258.0	17.0	539.3	25.1
		2401.1	120.0	5.2	320.6	7.6
	R	7335.0	336.0	20.0	572.6	26.4
		2511.2	125.6	5.4	226.7	5.7
J3	L	8498.0	424.0	22.0	884.6	33.1
		2837.0	200.5	5.8	292.3	5.4
	R	8329.0	416.0	22.0	985.5	35.0
		2836.9	200.5	5.9	294.3	5.2

a=Mean.
b=Standard deviation.

TABLE XLIV

ANALYSIS OF VARIANCE FOR MUSCLE FIBER AREA IN THE LONGISSIMUS DORSI

Source	df	Mean Square	Observed Significance Level
Animal	5	23,583.127	
Side	1	859.207	.5792
Animal x Side	5	1,100.847	
Method	1	739,837.935	.0011
Side x Method	1	1,626.907	.3435
Animal x Method	5	11,865.447	
Animal x Side x Method	5	1,477.707	
Corrected Total	23	40,541.725	

TABLE XLV

ANALYSIS OF VARIANCE FOR MUSCLE FIBER AREA IN THE SARTORIUS

Source	df	Mean Square	Observed Significance Level
Animal	5	104,550.07	
Side	1	1,516.86	.5931
Animal x Side	5	4,573.30	
Method	1	1,523,793.62	.0028
Side x Method	1	40.56	.9310
Animal x Method	5	44,215.94	
Animal x Side x Method	5	5,243.94	
Corrected Total	23	100,794.23	

TABLE XLVI

ANALYSIS OF VARIANCE FOR MUSCLE FIBER AREA IN THE SEMITENDINOSUS

Source	df	Mean Square	Observed Significance Level
Animal	5	61,072.39	
Side	1	14,479.59	.0195
Animal x Side	5	1,254.79	
Method	1	1,209,561.10	.0010
Side x Method	1	5,875.01	.0922
Animal x Method	5	18,881.17	
Animal x Side x Method	5	1,372.47	
Corrected Total	23	71,426.95	

TABLE XLVII

ANALYSIS OF VARIANCE FOR MUSCLE FIBER AREA IN THE TRICEPS BRACHII, LATERAL HEAD

Source	df	Mean Square	Observed Significance Level
Animal	5	56,524.910	
Side	1	38.760	.9479
Animal x Side	5	8,631.210	
Method	1	977,155.970	.0008
Side x Method	1	326.344	.7105
Animal x Method	5	12,641.020	
Animal x Side x Method	5	2,131.934	
Corrected Total	23	59,876.802	

TABLE XLVIII

ANALYSIS OF VARIANCE FOR MUSCLE FIBER AREA

Source	df	Mean Square	Observed Significance Level
Animal	5	204,433.52	
Muscle	3	255,358.06	.0001
Animal x Muscle	15	13,765.66	
Side	1	3,827.90	.5211
Muscle x Side	3	4,355.51	.2087
Animal x Side	5	7,882.67	
Animal x Muscle x Side	15	2,559.16	
Method	1	4,374,102.78	.0007
Muscle x Method	3	25,415.28	.1325
Side x Method	1	35.28	.9341
Muscle x Side x Method	3	2,611.18	.2559
Animal x Method	5	52,591.84	
Animal x Muscle x Method	15	11,670.58	
Animal x Side x Method	5	4,983.73	
Animal x Muscle x Side x Method	15	1,747.44	
Corrected Total	95	74,071.45	

TABLE XLIX

CROSS PRODUCTS CORRELATION COEFFICIENTS FOR MUSCLE FIBER AREA BY COULTER COUNTER AND PHOTOMICROGRAPHIC TECHNIQUES IN THE LONGISSIMUS DORSI, SARTORIUS, SEMITENDINOSUS AND TRICEPS BRACHII, LATERAL HEAD

Source	df	Longissimus dorsi Correlation Coefficients	Sartorius Correlation Coefficients	Semitendinosus Correlation Coefficients	Triceps brachii Correlation Coefficients
Animal	5	0.37	0.70	0.74	0.88
Side	1	-1.00	1.00	1.00	-1.00
Animal x Side	5	-0.24	-0.12	-0.04	0.69
Corrected Total	11	0.33	0.64	0.70	0.84

TABLE L

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL NUCLEAR NUMBER IN THE LONGISSIMUS DORSI

Source	df	Mean Square	Observed Significance Level
Animal	5	4.1865413 E+19	
Side	1	2.2371031 E+18	.5142
Animal x Side	5	3.8712560 E+18	
Location	2	2.6721867 E+17	.7988
Side x Location	2	1.4494692 E+17	.8832
'A x L + A x S x L'	20	1.1622644 E+18	
READ (Ani Side Loc)	36	1.1813073 E+17	
Corrected Total	71	3.6513067 E+18	

TABLE LI

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL NUCLEAR NUMBER IN THE SARTORIUS

Source	df	Mean Square	Observed Significance Level
Animal	5	3.5191356 E+17	
Side	1	2.5265689 E+16	.2364
Animal x Side	5	1.4014672 E+16	
Location	2	1.0065673 E+16	.0853
Side x Location	2	2.6884141 E+15	.5062
'A x L + A x S x L'	20	3.6347270 E+15	
READ (Ani Side Loc)	36	3.3700257 E+15	
Corrected Total	71	2.9217331 E+16	

TABLE LII

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL NUCLEAR NUMBER IN THE SEMITENDINOSUS

Source	df	Mean Square	Observed Significance Level
Animal	5	1.2167954 E+19	
Side	1	2.5180431 E+16	.7217
Animal x Side	5	1.7996577 E+17	
Location	2	3.0436667 E+17	.0775
Side x Location	2	1.7542481 E+17	.2129
'A x L + A x S x L'	20	1.0525047 E+17	
READ (Ani Side Loc)	36	5.7842129 E+16	
Corrected Total	71	9.4241818 E+17	

TABLE LIII

ANALYSIS OF VARIANCE FOR ESTIMATED TOTAL NUCLEAR NUMBER IN THE TRICEPS BRACHII, LATERAL HEAD

Source	df	Mean Square	Observed Significance Level
Animal	5	2.2886255 E+18	
Side	1	2.2439135 E+16	.5104
Animal x Side	5	3.9545485 E+16	
Location	2	1.1677188 E+16	.6435
Side x Location	2	2.1715892 E+16	.5560
'A x L + A x S x L'	20	2.5439580 E+16	
READ (Ani Side Loc)	36	6.1412530 E+15	
Corrected Total	71	1.7549235 E+17	

TABLE LIV

ANALYSIS OF VARIANCE FOR PROTEIN TO DNA RATIO IN THE LONGISSIMUS DORSI

Source	df	Mean Square	Observed Significance Level
Animal	5	12,222.1430	
Side	1	351.8489	.3204
Animal x Side	5	288.2811	
Location	2	584.3965	.3802
Side x Location	2	18.0166	.9697
'A x L + A x S x L'	20	572.8708	
READ (Ani Side Loc)	36	81.4424	
Corrected Total	71	1,105.6076	

TABLE LV

ANALYSIS OF VARIANCE FOR PROTEIN TO DNA RATIO IN THE SEMITENDINOSUS

Source	df	Mean Square	Observed Significance Level
Animal	5	22,903.1866	
Side	1	0.2100	.9884
Animal x Side	5	1,813.6964	
Location	2	2,193.9404	.0416
Side x Location	2	568.2496	.5984
'A x L + A x S x L'	20	591.2267	
READ (Ani Side Loc)	36	367.0513	
Corrected Total	71	2,171.0900	

TABLE LVI

ANALYSIS OF VARIANCE FOR PROTEIN TO DNA RATIO
IN THE TRICEPS BRACHII, LATERAL HEAD

Source	df	Mean Square	Observed Significance Level
Animal	5	17,068.6663	
Side	1	41.0675	.8601
Animal x Side	5	1,313.5534	
Location	2	73.6003	.8144
Side x Location	2	292.3074	.5468
'A x L + A x S x L'	20	351.4497	
READ (Ani Side Loc)	36	132.2318	
Corrected Total	71	1,471.4552	

TABLE LVII

CROSS PRODUCTS CORRELATION COEFFICIENTS FOR COULTER COUNTER MUSCLE FIBER AREA BY PROTEIN TO DNA RATIO

Source	df	Longissimus dorsi Correlation Coefficients	Semitendinosus Correlation Coefficients	Triceps brachii Correlation Coefficients
Animal	5	0.87	0.71	0.14
Side	1	1.00	1.00	1.00
Animal x Side	5	0.90	0.20	-0.09
Corrected Total	11	0.87	0.66	0.11

TABLE LVIII

CROSS PRODUCTS CORRELATION COEFFICIENTS FOR PHOTOMICROGRAPHIC MUSCLE FIBER AREA BY PROTEIN TO DNA RATIO

Source	df	Longissimus dorsi Correlation Coefficients	Semitendinosus Correlation Coefficients	Triceps brachii Correlation Coefficients
Animal	5	0.02	0.57	0.49
Side	1	-1.00	1.00	-1.00
Animal x Side	5	-0.43	-0.67	0.51
Corrected Total	11	-0.03	0.50	0.49

TABLE LIX

CROSS PRODUCTS CORRELATION COEFFICIENTS FOR COULTER COUNTER MUSCLE FIBER NUMBER BY TOTAL NUCLEAR NUMBER

Source	df	Longissimus dorsi Correlation	Sartorius Correlation	Semitendinosus Correlation	Triceps brachii Correlation
Animal x Side	5	0.53	-0.59	-0.13	0.15

TABLE LX

CROSS PRODUCTS CORRELATION COEFFICIENTS FOR PHOTOMICROGRAPHIC MUSCLE FIBER NUMBER BY TOTAL NUCLEAR NUMBER

Source	df	Longissimus dorsi Correlation	Sartorius Correlation	Semitendinosus Correlation	Triceps brachii Correlation
Animal x Side	5	-0.04	-0.54	-0.22	0.38

TABLE LXI

CORRECTION FACTOR FOR ANGLE (θ)

Angle (θ)	Correction Factor
0	1.00000
5	1.00127
15	1.01162
25	1.03333
35	1.06871
45	1.12220
55	1.20241
65	1.32790
75	1.54897
85	2.11071

VITA -2

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Master of Science

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